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Leaf Ethanolic Extract of *Bauhinia monandra* Increases Insulin Secretion in Rats Subjected to Intrauterine Malnutrition

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Authors' contributions

This work was carried out in collaboration between all authors. Authors ADOP and LCBBC designed the study, wrote the protocol and supervised the work. Authors ACCA and ACRDS carried out all laboratory work and performed the statistical analysis. Author VCRD managed the analyses of the study. Authors JBN and LCBBC wrote the first draft of the manuscript. Author AFSS managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This work aimed to investigate the effects of *Bauhinia monandra* ethanolic extract (E) on blood glucose levels (G), insulin and lipids of control (C) and prenatal malnourished (M) rats. **Samples:** Animals (from 90 days) were on standard or isocaloric high glucose diet (67%, w/w) during 30 days.

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Study Design: Animals received 0.1% DMSO (2.5 ml/kg, po.) or E (500 mg/kg, po.). Glucose tolerance was evaluated in blood following glucose overload (1.5 g/kg, po.).
Place and Duration of Study: Departamento de Bioquímica and Departamento de Fisiologia e Farmacologia, Universidade Federal de Pernambuco, between January 2006 and February 2007.
Methodology: In acute tests, glucose tolerance and serum insulin were evaluated in C and M receiving DMSO or E. Lipids and hepatic enzymes were analyzed before DMSO or E application. Glucose was orally administered by gavage, for glucose tolerance; thirty minutes later, E or DMSO was given. Blood samples were collected for glucose and insulin measurements. In the chronic assay, glucose tolerance, lipids and hepatic enzymes were studied in groups C or M receiving DMSO or E. The group M receiving E had a diet rich in glucose (30 days). Glucose tolerance, lipids, and hepatic enzymes were evaluated before and after diet rich in glucose treatment with E. Serum insulin was measured by chemiluminescence; also, aspartate and alanine aminotransferases were analyzed after high dietary glucose. Serum total cholesterol and triglycerides were estimated with an auto analyzer.
Results: In control rats, acute E administration reduced G, but did not change insulin secretion; in M rats, it induced insulin peak. In chronic study, animals on high glucose diet with E showed lower G; prenatal malnourished rats revealed higher insulin levels. Cholesterol, triglycerides and alanine aminotransferase did not change by intrauterine malnutrition or E.
Conclusion: Results indicated that E may act on beta-cells and stimulate insulin secretion.

Keywords: Bauhinia monandra; leaf ethanolic extract; hypoglycemic activity; blood glucose; serum insulin.

ABBREVIATIONS

ALT: Alanine Aminotransferases; AST: Aspartate Aminotransferases; BmoLL: Bauhinia Monandra Leaf Lectin; BmoRoL: B. Monandra Root Lectin; C: Control; E: B. Monandra Ethanolic Extract; G: Blood Glucose Levels; GCE: Group Control Receiving a Diet Rich in Glucose and Treated with Extract; Group CE: Control Receiving B. Monandra Ethanolic Extract; Group CV: Control Receiving Vehicle; GCV: Group Control Receiving a Diet Rich in Glucose and Treated with Vehicle; GME: Group of Prenatal Malnourished Wistar Rats Receiving a Diet Rich in Glucose and Treated with Extract; Group ME: Prenatal Malnourished Wistar Rats Receiving B. Monandra Ethanolic Extract; Group MV: Prenatal Malnourished Wistar Rats Receiving Vehicle; GMV: Group of Prenatal Malnourished Wistar Rats Receiving a Diet Rich in Glucose and Treated with Vehicle ; GTT: Glucose Tolerance; HDL: High Density Lipoprotein; M: Prenatal Malnourished Wistar Rats; NIDDM: Non-insulin Dependent Diabetes Mellitus; V: Vehicle.

1. INTRODUCTION

Plants from the genus *Bauhinia* are widely distributed in most countries and have been frequently used in folk medicine to treat several ailments, including diabetes [1,2,3]. In recent years, with the higher incidence of diabetes in the world, the interest in these plants has increased considerably [4,5]. Medicinal plants have been an alternative for treatment of diabetes in developing countries. There are more than 350 traditional plants already used with this aim [6] and the antidiabetic activity is mainly due to presence of the secondary metabolite in plants [7]. Therefore, active compounds of these plants may be used to develop new drugs for treatment of diabetes. The therapeutic properties of *Bauhinia* have been supported by studies using plant extracts [8,2]. Aqueous, ethanolic and hexanic extracts [2,4] from *B. forficata* leaves have induced hypoglycemia in rats. The *Bauhinia* genus presents terpenes, steroids, lactones and flavonoids [8]. *B*. *monandra* Kurz ("pata-de-vaca", "orquidea del pobre", pulse or "Napoleon's plume") is an ornamental Fabaceae species with an expressive content of galactose specific proteins, namely *B*. *monandra* leaf lectin, BmoLL [1] and *B*. *monandra* root lectin, BmoRoL [9]. BmoLL showed hypoglycemiant activity without cytotoxic or genotoxic effects [10]. In Northeastern Brazil, the leaves of *B. monandra* are largely used in folk medicine to reduce blood glucose levels.

In this way, type 2 diabetes mellitus previously called non-insulin dependent diabetes mellitus (NIDDM) or maturity-onset diabetes is a combination of tissue resistance to insulin action

with an abnormal insulin secretion in response to glucose [6] and beta cell dysfunction, strongly associated with obesity and a sedentary lifestyle [11]. Since 2010, it was estimated that 280 million of people had diabetes, with NIDDM making up about 90% of the cases globally. The incidence of this disease is increasing rapidly and at the end of 2030, the number of cases will be double, because of increasing longevity and obesity [6]. Diabetes is an important problem to health policy in all world and its prevalence is on a steady increase worldwide. This disease is now identified as one of the main threats to human health in the 21st century [11] considering its high incidence and chronic complications, such as coronary heart disease [12], nephropathy, retinopathy [13], chronic kidney disease [14], renal failure, blindness, amputations and hospitalizations [15].

Also, glucose intolerance and hyperinsulin can be induced by prenatal malnutrition [16]. Furthermore, when prenatal malnutrition is experimentally induced in rats, oligonephronia [17,18] and its consequences such as hypertension, renal hemodynamic and glomerular morphological changes [17] have been observed. The factors above are compatible with the development of chronic renal failure, commonly accompanying diabetes. In accordance with these findings, prenatal malnutrition and low birth weight in humans have been correlated with renal disease during adult life [19].

Currently available oral agents for the treatment of type 2 diabetes mellitus include a variety of compounds from five different pharmacologic classes with distinct mechanisms of action: the sulfonylureas, metiglinides, biguanides, α-glucosidase inhibitors, and thiazolidinediones [12]. In general, α-glucosidase inhibitors delay carbohydrate absorption, metiglinides and sulfonylureas increase insulin supply; biguanides and thiazolidinediones enhance insulin action [12]. However, these drugs have limited use because of pathogenically undesirable conditions and high rates of secondary failure [6].

The aim of this work was to investigate the acute and chronic effects of the ethanolic extract from *B. monandra* leaves to treatment of rats subjected to intrauterine malnutrition. The use of this plant tissue in the popular medicine to diabetes treatment was the main motivation of the present study. The extract effects were evaluated on blood glucose and insulin in rats; lipids and hepatic enzymes were also investigated.

2. MATERIALS AND METHODS

2.1 Preparation of the Extracts

All chemicals were of analytical grade. Solvents for the extraction were purchased from Merck (Darmstadt, Germany). Leaves of *B. monandra* Kurz were harvested from ornamental trees in Recife (State of Pernambuco, Northeast of Brazil). A sample of the collected material is archived as voucher specimen number 57462, at the herbarium Dárdano de Andrade Lima, Empresa Pernambucana de Pesquisa Agropecuária, Recife City, Brazil. Dried and powdered leaves of *B. monandra* (200 g) were extracted with 95% ethanol (1 L) until exhaustion. After filtration, the combined extracts were concentrated at 40ºC to dryness, the ethanolic extract (E).

2.2 Diets

Intrauterine malnutrition was induced using a multideficient diet, as previously described [20,17]. The content of its main nutrients was (g/g%): proteins 9, carbohydrates 78, lipids 1.1, fiber 7, minerals 4, sodium chloride 0.17 and Kcalories 356. No vitamin supplement was added. However, part of the diet was supplemented with 0.2% (g/g) sodium chloride. The same nutrient in the standard diet (as indicated by the manufacturer, Purina, Agribands do Brasil, Paulínia, SP, Brazil) contains the following contents (g/g%): protein 23, carbohydrates 41, lipids 2.5, fibers 9, minerals 8, sodium chloride 0.37 and Kcalories 278.

The content of the diet high in glucose was (g/g%): glucose 66.73, standard diet 33.27 (Purina, Agribands do Brasil, Paulínia, SP, Brazil) to obtention of an isocaloric diet.

2.3 Animals

All experiments were performed on control (C) and intrauterine malnourished (M) Wistar rats of both genders (250-300 g). Animals were kept under a constant 12 h light and dark cycle and an environmental temperature of 23ºC. Rats, C and M were obtained of dams on a standard or on a multi deficient diet, respectively, during mating and pregnancy. After parturition, all rats had free access to food standard diet and water. At adult age, part of the rats was maintained from 13-16 weeks on a standard diet, the groups C and M

and another part was maintained for the same period on a diet high in glucose (G), the groups GC and GM. All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the the Ethic Committee from the Centro de Ciências Biológicas, Universidade Federal de Pernambuco (document number 35/05).

2.4 Experimental Protocol

The biological assays were performed into two series, the acute test and the chronic test. In the first series, glucose tolerance test (GTT), and serum insulin were evaluated in C and M receiving vehicle (V, 0.1% DMSO), the groups CV (n=8) and MV (n=8); and in C and M receiving E. (500 mg/kg, po.), the groups CE (n=8) and ME (n=7). Additionally, lipids and hepatic enzymes were studied, before V or E was administered in all animals. Animals were fasted for 12 h to collect blood samples for biochemical measurements. Blood withdrawal was performed from retro-orbital plexus in the animals under ether anesthesia. Glucose (1.5 g/kg) was orally administered by gavage, for GTT study, after a basal blood sample collection. Thirty minutes later, E or V (2.5 mL/kg, po.) were administrated, also by gavage. Subsequently, blood samples were collected after 1, 2 and 4 h, for glucose and insulin measurements.

In the second series, GTT, lipids and hepatic enzymes were studied in GCV (n=9), GMV (n=13), GCE (n=9) and GME (n=13) receiving a diet rich in glucose and simultaneously treated with E (500 mg/kg, po) or V (2.5 mL/kg, po), during 30 days. GTT, lipids, and hepatic enzymes were evaluated before and after diet or E treatment. Body weight, food intake and blood glucose levels were monitored weekly during 30 days of treatment.

2.5 Analytical Methods

Blood glucose levels were determined by the glucose oxidase method [21] immediately after collection. Assay of serum insulin in blood was measured by chemiluminescence (Beckman Coulter™, Access®, USA). Serum activities of aspartate and alanine aminotransferases, AST and ALT, respectively, were measured by the kinetic method [22] after high dietary glucose. Serum total cholesterol and triglycerides were estimated by enzymatic method with an autoanalyzer using commercial reagents.

2.6 Statistical Analysis

Data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was evaluated by using one way ANOVA, followed by Student-Neuman-Keuls test to determine the significant difference between the groups. *P* values of = .05 were considered to be statistically significant.

3. RESULTS AND DISCUSSION

3.1 Body Weight

Prenatal malnutrition was based on the low birth weight from offspring of dams submitted to multideficient diet [23]. Higher basal serum glucose and insulin levels as well as alterations in glucose homeostasis after an acute glucose overload indicated that this was a valuable model to study hypoglycemic activity in leaf extracts from *B. monandra*. The body weight at birth was 19% lower in M than in C rats (5.2±0.2 vs. 6.4±0.08 g, p = .05, respectively), while at adult age the values of body weight were the same for both groups (364±24 vs. 323±19 g, respectively). In the groups chronically treated with E, basal values of body weight were similar for both GC and GM groups; at the end of E administration there was a not significant tendency of body weight loss in both GC and GM groups (Fig. 1).

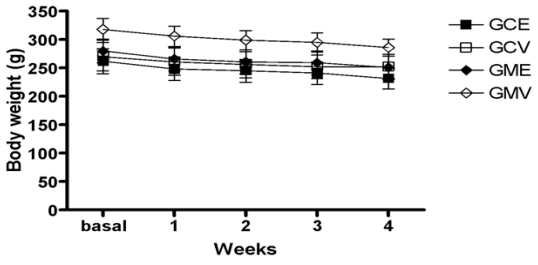

Fig. 1. Effect of leaf ethanolic extract from *B. monandra* on body weight of control, GCE (▪), GCV (▪), and prenatal malnourished rats, GME (◆) and GMV (◇) for 4 weeks.

Values are given as mean ±S.E. n=7/8

The present findings corroborate with previous studies showing glucose intolerance [19]. Furthermore, the prenatal malnutrition is also a condition in which growth factors occur in serum [24] that may contribute with atherosclerotic

disease, a common chronic complication present in non-insulin dependent diabetes mellitus [25].

3.2 Acute and Chronic Effect of E on Serum Glucose and Insulin Levels

In the acute study, the effect of E on serum glucose levels is shown in Figs. 2 (A and B).

Basal fasting serum glucose was higher (18%, *p* = .05) in M than in C rats (122±4 vs. 103±6 mg/dL, respectively). Both CV and MV presented a glucose peak in the first hour after glucose overload (188±6 and 171±8 mg/dL, respectively). During the peak, the glucose level was lower in CE and ME as compared with their respective control groups (161±7 and 156±5 mg/dL, respectively). In the fourth hour after V or E administration, the serum glucose levels returned to their basal values in all groups. Basal serum insulin in CV was lower than in MV rats (0.32±0.11 vs. 2.17±0.82 ⌈IU/mL, *p* = .05, respectively, Fig. 2 C and D). Different from CV, the CE group did not show a peak of insulin secretion in the first hour after glucose overload (1.36±0.45 vs. 0.52±0.26 ⌈IU/mL, respectively). However, ME presented a peak of insulin secretion in the first hour (1.93±1.03 ⌈IU/mL), while CE did in the second hour after glucose overload (1.40±0.57 ⌈IU/mL). Acute E administration reduced the glucose level in C rats, however the peak of insulin secretion in CE rats was retarded to 2 h (Fig. 2 C).

Serum glucose evaluated weekly during chronic treatment was found to be reduced in GC and GM rats (p = .05, Fig. 3).

At the end of treatment, during GTT evaluation, basal fasting serum glucose was higher in GMV and GCV groups than that seen in GME and GCE groups, respectively (137±4 vs. 100±5 mg/dL and 119±5 vs. 94±5 mg/dL, respectively; p = .05, Figs. 4A and B). Also, serum glucose was high in GMV than in GCV group in the first and fourth hour, respectively (205±6 vs. 184±4 mg/dL and 144±4 vs. 125±4 mg/dL, respectively; p = .05, Figs. 4A and 4B). In the two groups chronically treated with E, GME and GCE, the serum glucose peak after acute glucose overload was lower than that in their respective control groups, GMV and GCV (164±8 vs. 205±6 and 153±6 vs. 184±4 mg/dL, respectively; p = .05, Fig. 4 A and B).

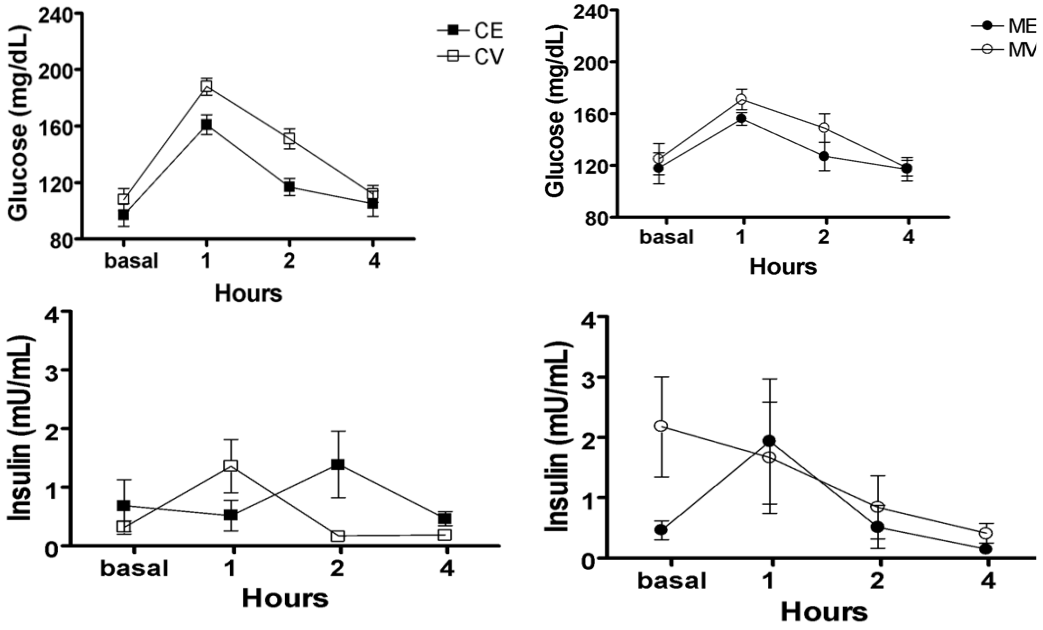

Fig. 2. Acute effect of leaf ethanolic extract from *B. monandra* on serum glucose (A and B) and insulin (C and D) levels of control (CE and CV, n=8) and prenatal malnourished (ME, n=7 and MV, n=8) rats. CE (▪), CV (▪), ME (▪) and MV (○)

*Values are given as mean ±S.E. * P = .05 (CE vs. CV), # P =.05 (ME vs. MV).*

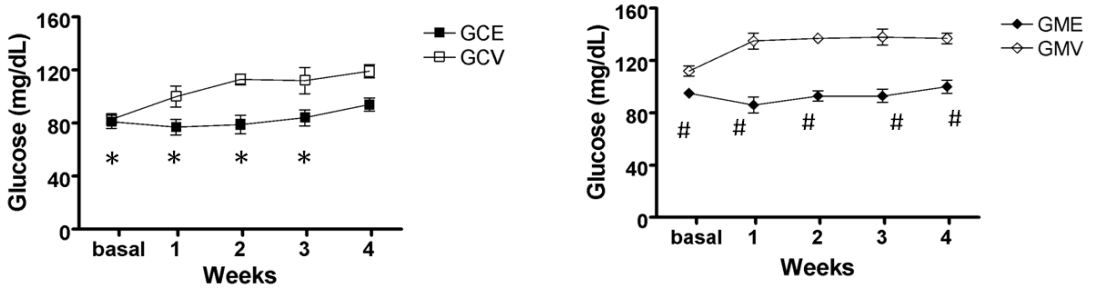

Fig. 3. Effect of leaf ethanolic extract from *B. monandra* on weekly glucose level of control (A; GCE and GCV, n=9) and prenatal malnourished (B; GME and GMV, n=13) rats. GCE (▪), GCV (▪), GME (◆) and GMV (◇)

*Values are given as mean ±S.E. * P = .05 (GCE vs. GCV), # P = .05 (GME vs. GMV)*

At the end of treatment, during GTT evaluation, basal level of serum insulin was similar for both groups, GME and GCE rats (0.89±0.31 µIU/mL, and 0.51±0.08 µIU/mL, Figs. 4C and 4D). However, at the first hour, the levels of serum insulin were higher in GME than in GMV rats (0.44±0.11 µIU/mL vs. 0.167±0.032 µIU/mL, respectively; p = .05). Different from GCE, GME group did not show a peak of insulin secretion in the first hour after glucose overload (1.64±0.43 vs. 0.44±0.11 µIU/mL, respectively; p = .05). The values of serum insulin peak were similar for both groups GCE and GCV. Although, neither GMV nor GME had presented an insulin peak, GME presented higher insulin secretion during all points of glucose tolerance curve. Thus, E seemed to restore the capacity of M rats to secrete insulin.

It was reported by Lino et al. [2] that aqueous, ethanolic and hexanic extracts of *Bauhinia forficata* leaves showed antidiabetic activity; so, this plant extracts can be used in the treatment of diabetes mellitus type II. Antihyperglycemic activity was also revealed when it was used aqueous extract of fresh leaves of *B. forficata* (1 g/kg, p.o.) [4]. In our study, based on the hypoglycemic effect of E on control and prenatal malnourished rats, and on the levels of plasma insulin, it may be hypothesized that the mechanisms involved include an augmentation on insulin secretion, like the sulfonylureas, an oral agent for the treatment of type 2 diabetes mellitus compound, lower blood glucose in normal and type 2 diabetic animals by stimulating insulin release from pancreatic cells [6].

These findings are in agreement with hypoglycemic activity induced by extracts from other *Bauhinia* species, such as *B. candicans, B. forficata*, *B. variegata* and *B. tomentosa* [2,3,5].

3.3 Profile of Cholesterol, Triglycerides, AST and ALT in Control and Prenatal Malnourished Rats

Plasma levels of AST and ALT, withdrawn before V or E administration in the acute study, were similar for both groups, M (150±10.5 and 71±2 IU/L, respectively) and C (164±8 and 59±5 IU/L, respectively), while the levels of total cholesterol and triglycerides, also withdrawn before V or E administration in the acute study, were higher in C (100.4±5.3 and 254±37 mg/dL, respectively; p<0.05) than M (77.8±7 an *B. variegata* leaf ethanolic extractd 140±15 mg/dL, respectively; p<0.05). Table 1 shows the effect of chronic E treatment on the plasma levels of AST, ALT, total cholesterol and triglycerides. It was remarkable that AST was lower in GMV than the one observed in GCV rats (139±8 vs. 187±9 IU/L, respectively; p< 0.05). Regarding E effects, the group GME presented AST 55% higher (p < 0.05) than that one seen in GMV.

Thiruvenkatasubramaniam and Jayakar [3] and Aljobouri et al. [5] studied the hypoglycemic effect of *Bauhinia variegata* leaf ethanolic and aqueous extract and there was significant reduction in total cholesterol, triglycerides and elevation of high density lipoprotein (HDL) in diabetic mice. Also, Lino et al. [2] study showed reductions in plasma glucose, triglycerides, total cholesterol and HDL-cholesterol in a model of alloxan-induced diabetes in rats treated by extracts of *B. forficata.*

Table 1. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglycerides and cholesterol levels in the serum of control (C) and malnourished (M) rats treated with vehicle (V) and extract (E)

	GCE (n=9)	GCV (n=9)	GME (n=13)	GMV (n=13)
AST (IU/L)	205±15	187±9.4 #	216.7±13.4#	139.1±7.6
ALT (IU/L)	74.7±6	70.1±3.8	73.5±4.3	70.1±1.9
Triglycerides (mg/dL)	186±34.1	188.4±34.5	122.3±16.4	129.6±13.6
Cholesterol (mg/dL)	82.3±6.8	90±4.7	80±4.7	84.2±4.8

Results are expressed as means ± SEM. # P = .05 (vs. GMV). n=7/8. GCE: Group control receiving a diet rich in glucose and treated with extract; GCV: Group control receiving a diet rich in glucose and treated with vehicle; GME: Group of prenatal malnourished Wistar rats receiving a diet rich in glucose and treated with extract; GMV: Group of prenatal malnourished Wistar rats receiving a diet rich in glucose and treated with vehicle

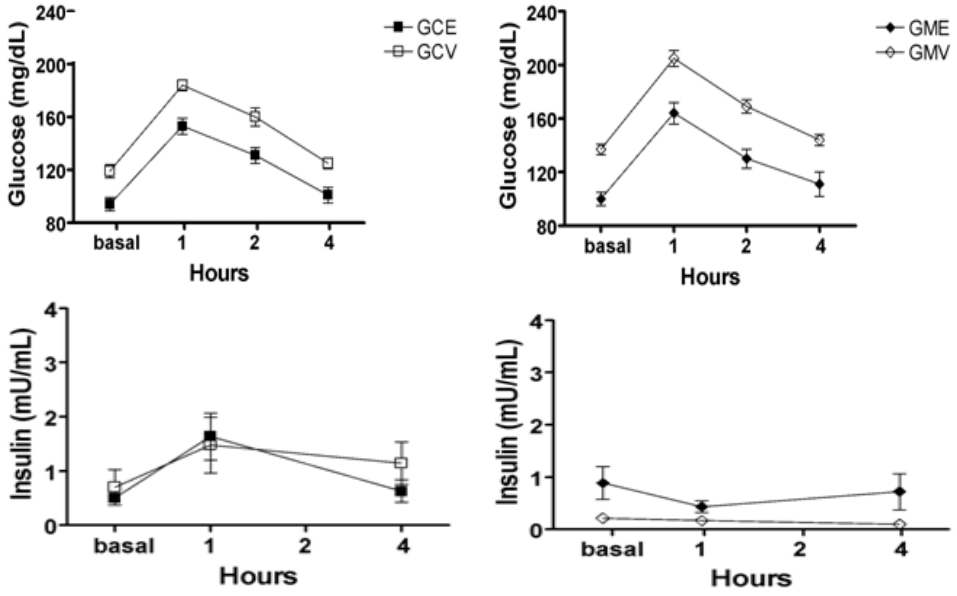

Fig. 4. Chronic effect of leaf ethanolic extract from *B. monandra* on serum glucose (A and B) and insulin (C and D) level of control (GCE and GCV, n=9) and prenatal malnourished (GME and GMV, n=13) rats. GCE (▪), GCV (▪), GME (◆) and GMV (◇)

*Values are given as mean ±S.E. * P = .05 (GCE vs. GCV), # P = .05 (GME vs. GMV)*

4. CONCLUSIONS

The results obtained in this work provide evidence for a direct pancreatic action of *B. monandra* leaf ethanolic extract, probably through an activity on endocrine pancreatic β cells. This is an innovative study that reports for the first time that a hypoglycemic effect of *B. monandra* leaf ethanolic extract may act on beta-cells and stimulate insulin secretion. The property should be explored with good prospects for pharmacological application.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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