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Serum and liver lipids distributions in streptozotocin induced diabetic rat treated with diet containing Yam (Dioscorea bulbifera) flour

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Abstract

Introduction: **Liver disease as a major cause of mortality in patients with diabetes mellitus. There is a interest to investigate the hypolipidemic properties of yam. The goal was assess the role of Brazilian yam (Dioscorea bulbifera) on serum and hepatic levels of triglycerides and cholesterol, in female diabetic rats.**

Methods: **The rats were divided into three groups: Control (C), Diabetic (DM); Diabetic Yam (DMY), treated with diet containing 25g/100g of yam flour. After 5 weeks of experiment, glucose, insulin, gonadal fat and liver mass were evaluated. Serum and liver concentrations of triglycerides and cholesterol concentrations were quantified. Total liver thiols were determined.**

Results: **After the 5 weeks, experimental groups shower (P < 0.05): Lower body mass; lower serum insulin; higher food intake and higher blood glucose concentration. DMY (vs. DM) group showed (P < 0.05): Lower blood glucose; higher gonadal fat mass; lower serum and hepatic triglycerides; higher hepatic cholesterol and thiols concentrations. DMY (vs. C) group showed: Similar serum and hepatic triglycerides and hepatic thiols.**

Conclusions: **Brazilian yam (Dioscorea bulbifera) alleviated the consequences of the experimental diabetic disease, suggesting protection to hypertriglyceridemia and lipid peroxidation.**

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Key words: *Dioscorea bulbifera. Lipid profile. Thiol. Streptozotocin. Diabetic rats.*

SUERO Y HEPÁTICAS LÍPIDOS EN RATAS DIABÉTICAS TRATADOS CON DIETA QUE CONTENÍA HARINA ÑAME (DIOSCOREA BULBIFERA)

Resumen

Introducción: **La enfermedad hepática como una de las principales causas de mortalidad en los pacientes con diabetes mellitus. Hay un interés para investigar las propiedades hipolipemiantes de ñame. El objetivo era evaluar el papel de ñame brasileña (Dioscorea bulbifera) en suero y los niveles hepáticos de triglicéridos y colesterol, en las ratas diabéticas .**

Métodos: **Las ratas se dividieron en tres grupos: control (C), diabéticos (DM); Diabetic ñame (DMA), se trata con dieta que contenía 25 g / 100 g de harina de ñame. Después de 5 semanas de experimento, la glucosa, la insulina, la grasa gonadal y la masa del hígado fueron evaluados. Se cuantificaron las concentraciones de suero e hígado de los triglicéridos y las concentraciones de colesterol. Se determinaron los tioles totales de hígado.**

Resultados: **Después de las 5 semanas, experimental ducha grupos (p <0,05): la masa corporal inferior; insulina sérica inferior; más alta la ingesta de alimentos y una mayor concentración de glucosa en sangre. DMY (vs. DM) grupo mostró (P <0.05): Baje la glucosa en sangre; masa grasa gonadal superior; inferior y triglicéridos séricos hepáticas; más altos de colesterol y tioles concentraciones hepáticas. DMY (vs. C) grupo mostró: suero similares y triglicéridos hepáticos y tioles hepáticas.**

Conclusiones: **Ñame brasileño (Dioscorea bulbifera) alivian las consecuencias de la enfermedad diabética experimental, lo que sugiere protección para la hipertrigliceridemia y la peroxidación lipídica.**

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Palabras clave: *Dioscorea bulbifera. Perfil lipídico. Tiol. Estreptozotocina. Ratas diabéticas.*

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Introduction

Diabetes mellitus (DM) is a chronic disease characterized by hyperglycaemia as the result of defects during the production, secretion or action of insulin.[1] Defect in glucose-regulating endocrine tissues or dysregulation of genes involved in the metabolism and transport of carbohydrate, protein, and lipids are key factors precipitating diabetes.[2] Previous clinical studies documented liver disease as a major cause of mortality in patients with DM.[3,4] The scope of liver disease in DM includes the non-alcoholic fatty liver disease (NAFLD), characterized by fat accumulation in hepatocytes. Conditions like hypertriglyceridemia and hypercholesterolemia are described as a cause of NAFLD.[5] Several organizations, recommend that lifestyle modifications, such nutrition therapy, has been shown to help some patients to achieve better lipid levels.[6]

In the context, our group reported the effects of Brazilian yam (*Dioscorea bulbifera*) in female diabetic rats. The perennial herb alleviated the consequences of the experimental diabetic disease, with abbreviation of hyperglycaemia.[7,8] Furthermore, the animals showed lower risk to gastrointestinal disorder,[7] common complication between diabetic patients.

The Dioscorea is employing for the treatment of several diseases, such as diabetes, in traditional Chinese medicine.[9] Yam (Dioscorea spp.), a perennial herb, is distributed mainly in the tropical and subtropical regions of the world.[10] Although little attention has been given to the physicochemical and phytochemical properties of Dioscoreaceas, especially in Brazil,[11] the herbal medicines have been known to embrace minimal side effects and toxicity.[12]

Thus, the present study was designed to evaluate the repercussion of diet, containing Brazilian yam (*Dioscorea bulbifera*) flour, upon serum and hepatic levels of triglycerides and cholesterol, in female diabetic rats.

Materials and Methods

Tubers of yam *(Dioscorea bulbífera)* were purchased from a local market in Belo Horizonte (MG, Brazil) and categorized by a biologist in comparison to the existing voucher specimen in Jardim Botânico (RJ, Brazil): Couto, R.S. 164.

The protocol to use and handling the experimental animals was approved by the Animal Ethics Committee of the Federal Fluminense University (n 37, 2010). The studied was conducted in Female Wistar rats with 3 months age, weighting about 190 – 200 g. The animals maintained under controlled room temperature (22 ± 2 °C) with 12h light/12h dark cycle, fed a normal chow diet (Nuvital®, Brazil) and water *ad libitum*, prior to the dietary manipulation.

The rats were allocated into two dietary regimens by feeding either Normal Diet (10% lipids, 12% protein and 78% de carbohydrate as a percentage of total kcal), according to *American Institute of Nutrition* (AIN) recommendations,[13] or High Fat Diet (HFD, 60% lipids, 14% protein and 26% de carbohydrate as a percentage of total kcal) *ad libitum*, for 3 weeks. Then, HFD rats were injected intraperitoneally (i.p.) with streptozotocin (STZ, 35mg/kg), while the respective control rats were given vehicle citrate buffer (1mL/kg, i.p, pH 4.4).[14,15] After 1 week, the fasting glucose level of a blood obtained from the tail was measured using a glucometer and the rats with the glycemia of more than 290mg/dL were considered diabetic and selected for the study.

The rats were divided into three groups based on diet (Table I): Control group (C, n = 6 healthy rats) received an AIN-93M-based control diet; Diabetic group (DM, n = 8 diabetic rats) received a fat diet; Diabetic Yam group (DMY, n = 8 diabetic rats), treated with fat diet containing 25g/100g of yam flour. Each tuber of yam was washed free of dirt, skinned and then cut to 0.5 cm thick slices. The cut samples were boiled for 60 seconds, then freezed, lyophilized. The dried yam was milled to ensure homogeneity and then pass through a 35-mesh sieve and stored in plastic bags at -20°C until use.[16-18] The lyophilized yam powder contains (in g/100g): Moisture 3.62; ash 2.85; protein 7.33; fat 0.38; carbohydrates (starch + reducing sugar) 82.13.

The animals were given *ad libitum* access to their respective diets and water. During the 5 weeks of experiment, body mass and total food intake was evaluated weekly.

At the end of this period, after 6 h overnight fasting, blood samples were collected from the distal end of the tail and analyzed immediately with a glucometer and the corresponding test strips (Accu-Check Active®). Then the animals were anesthetized by Thiopentax® (Cristália, Rio de Janeiro, Brazil) followed by exsanguination. Blood was collected by cardiac puncture and samples were centrifuged, and serum was at -80°C for posterior analysis of triglycerides and cholesterol by colorimetric method (Bioclin, Belo Horizonte, MG, Brazil). Insulin concentrations were determined using multiplex assay kits (RADPK-81K, Millipore, Germany).

Gonadal fat depots and liver were dissected and the mass was expressed as total (g) and fractional ($g\ 100g^{-1}$) (adjusted to total body mass). Liver samples were used to analyses of hepatic lipids by the Haug & Hostmark[19] method. For each rat a 5% (wt/vol) homogenate in isopropanol was made of hepatic tissue. To extract triglycerides and cholesterol, the homogenate was kept at 4°C for 2 d, and then centrifuged for 10 min at 3000 rpm. Hepatic triglycerides and cholesterol were quantified in the aliquots of the supernatants by the same commercial kits used for serum lipid analyses.

Total liver thiols were determined in a spectrophotometer (Hitachi U-3300) using 5,5-dithionitrobenzoic acid (DTNB). Thiols react with DTNB, cleaving the disulfide bond to give 2-nitro-5thiobenzoate (NTB^-),

Table I
Composition of experimental diets

Ingredient (g per 100g)	*C*	*DM*	*DMY*
Casein	12.76	12.76	12.76
Cornstarch	63.31	45.79	20.79
Yam Flour	-	-	25.0
Sucrose	10.0	10.0	10.0
Soybean oil	4.0	4.0	4.0
Lard	-	17.51	17.51
Fiber	5.0	5.0	5.0
AIN-93M Mineral Mix	3.5	3.5	3.5
AIN-93 Vitamin Mix	1.0	1.0	1.0
L-Cystine	0.18	0.18	0.18
Choline Bitartrate	0.25	0.25	0.25
Energy			
Kcal g-1*	3.46	4.28	4.53
Protein (% of energy)*	12.00	10.00	10.00
Carbohydrate (% of energy)*	75.00	45.00	43.60
Fat (% of energy)*	13.00	45.00	45.00

Formulated to meet the American Institute of Nutrition AIN-93G recommendations for rodent diets. C, control group, fed a control diet; DM, diabetic group fed high fat diet; DMY, diabetic group fed high fat diet containing yam flour.
Casein: Farmos®; Cornstarch: Unilever Bestfoods®; Sucrose: União®; Soybean oil: Cargill®; Lard: Sadia®; Fiber: Blanver®; Mineral and Vitamin Mix: PragSoluções®; L-Cystine and Choline Bitartrate: M.Cassab®.
*Calculated from the amount of proteins, carbohydrates an lipids derived from casein, corn starch, yam flour, sucrose, soya bean oil and lard, respectively.

which ionizes to the NTB^{2-} di-anion in water at neutral and alkaline pH. The NTB^{2-} was quantified in a spectrophotometer by measuring the absorbance of visible light at 412 nm.[20]

Statistical analyses were carried out using the Graph Pad Prism statistical package version 5.0, 2007 (San Diego, CA, USA). Food intake and body mass were analyzed by two-way ANOVA, followed by Bonferroni post-test. The remaining results were analyzed by one-way ANOVA, followed by Newman-Keuls post-test and expressed as means ± SEM, with significance level of 0.05.

Results

During the 5 weeks of experiment period, the DM and DMY groups shower similar food intake, but these were significantly ($P < 0.05$) increased compared with the control group (Fig. 1A). In regard to body mass, the DM group showed lower ($P < 0.05$) development when compared with control group. The DMY showed intermediate body mass between the C and DM groups. But, at the end of the experimental period, the DM and DMY groups showed lower ($P < 0.05$) body mass compared with the control group (Fig. 1B).

After the 5 weeks of experiment, the DM and DMY groups shower lower ($P < 0.05$) total (C: 11.00 ± 1.20, DM: 3.36 ± 0.57 and DMY: 6.10 ± 1.06 g; Fig. 2A) and fractional (C: 3.47 ± 0.28, DM: 1.28 ± 0.19 and DMY: 2.05 ± 0.26 g $100g^{-1}$; Fig. 2B) gonadal fat mass compared with the control group. Moreover, the DMY group showed higher ($P < 0.05$) total and fractional gonadal fat mass compared with the DM group. The total liver mass did not differ between groups (C: 8.83 ± 0.52, DM: 9.03 ± 0.26 and DMY: 9.87 ± 0.32 g; Fig. 2C). However, the DM and DMY groups shower higher ($P < 0.05$) fractional liver mass (C: 2.81 ± 0.14, DM: 3.53 ± 0.09 g and DMY: 3.58 ± 0.20 g $100g^{-1}$; Fig. 2D) compared with the control group.

DM and DMY showed higher ($P < 0.05$) blood glucose concentrations compared with the C group (C: 183.70 ± 6.31, DM: 538.10 ± 16.08 and DMY: 480.70 ± 15.73 mg dL^{-1}). But, the DMY group showed lower ($P < 0.05$) blood glucose in regard to DM group (Fig. 3A). The experimental groups showed lower ($P < 0.05$) serum insulin concentrations compared with the C group (C: 81.56 ± 10.10, DM: 8.50 ± 2.52 and DMY: 6.59 ± 2.48 μIU mL^{-1}; Fig. 3B). The DM group showed higher ($P < 0.05$) serum triglycerides concentrations when compared to C and DMY groups (C: 65.60 ± 6.66, DM: 146.50 ± 18.52 and DMY: 68.67 ± 12.56

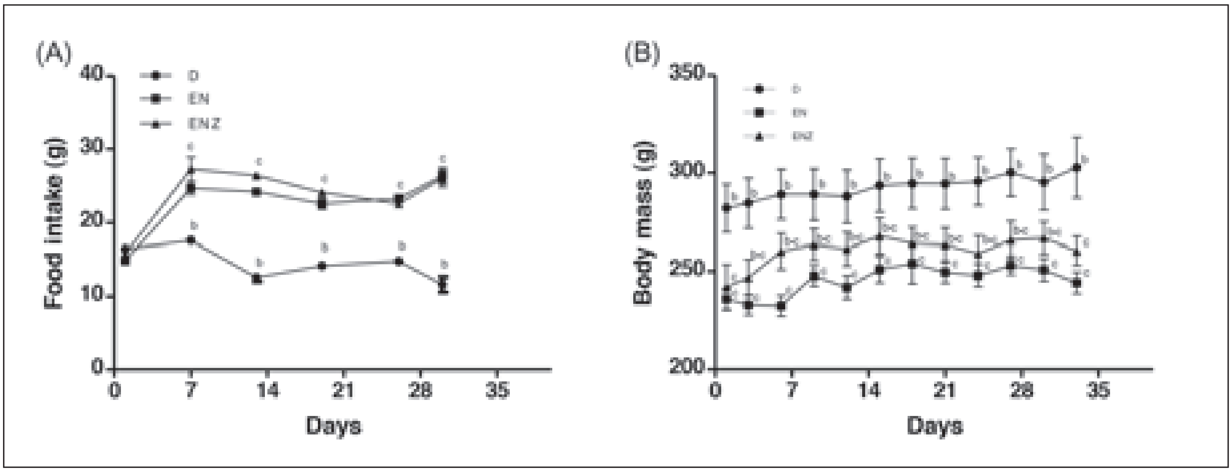

Fig. 1.—Food intake (A) and body mass (B) during the 5 weeks of experiment, respectively. C, control group (n=6); DM, diabetic group fed with high-fat diet (n=8); DMY, diabetic group fed with high-fat diet containing yam flour (n=8). Mean values with different superscripts (a and b) are significantly different. Two-way ANOVA, P < 0.05.

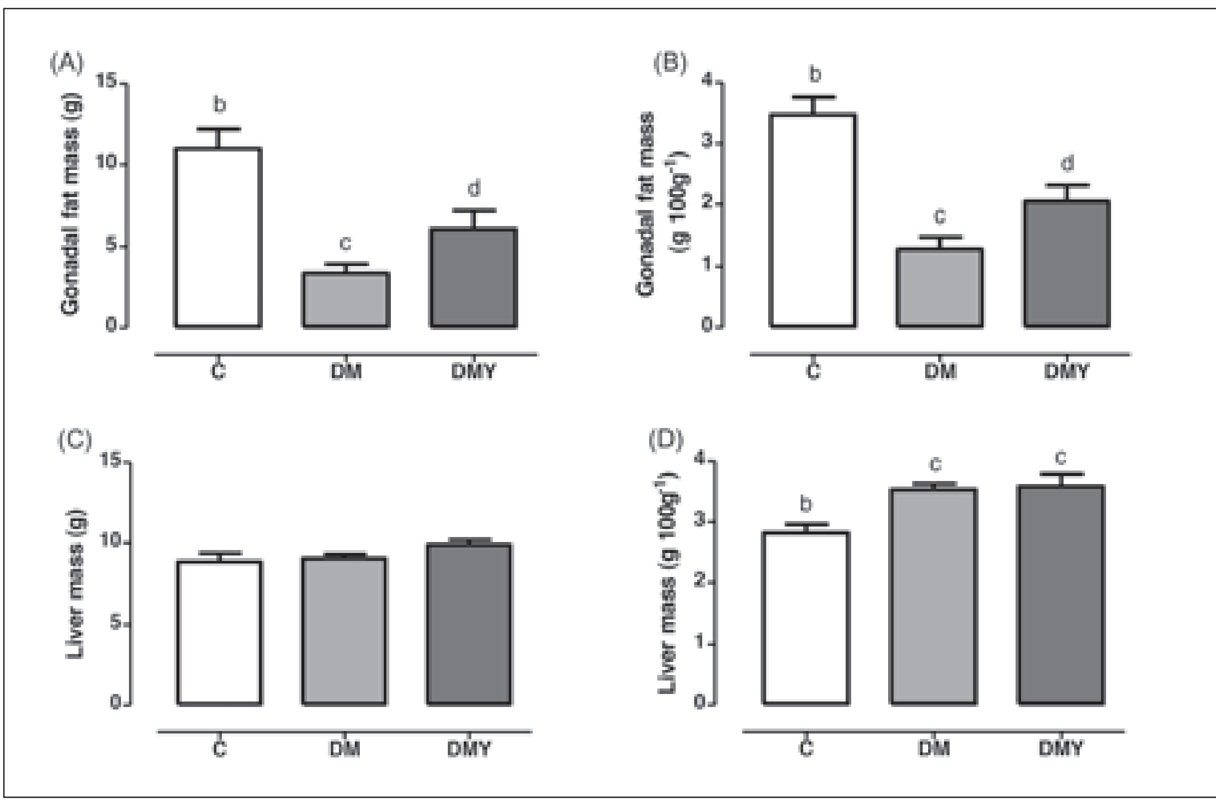

Fig. 2.—Gonadal fat and liver mass expressed as total (A and C) and fractional (B and D), after of the 5 weeks of experiment, respectively. C, control group (n=6); DM, diabetic group fed with high-fat diet (n=8); DMY, diabetic group fed with high-fat diet containing yam flour (n=8). Mean values with different superscripts (a, b and c) are significantly different. One-way ANOVA, P < 0.05.

mg dL^{-1}; Fig. 3C). Serum cholesterol concentrations did not differ between groups (C: 61.50 ± 4.20, DM: 75.57 ± 5.77 and DMY: 62.00 ± 6.73 mg dL^{-1}; Fig. 3D).

The liver analyses showed higher ($P < 0.05$) triglycerides concentrations in DM group compared with the C and DMY groups (C: 895.20 ± 89.31, DM: 2398.00 ± 253.90 and DMY: 1479 ± 151.20 mg dL^{-1}; Fig. 4A). DM and DMY groups showed higher ($P < 0.05$) liver cholesterol concentrations (C: 14.23 ± 0.88, DM: 47.41 ± 5.49 and DMY: 87.65 ± 4.32 mg dL^{-1}). But, the DMY group showed higher ($P < 0.05$) cholesterol concentrations compared with the DM group (Fig. 4B). The total liver thiols analyses showed lower ($P < 0.05$) concentrations in DM group compared with the C and DMY groups (C: 1.79 ± 0.10, DM: 0.79 ± 0.15 and DMY: 2.19 ± 0.21 mmol mg^{-1}; Fig. 4C).

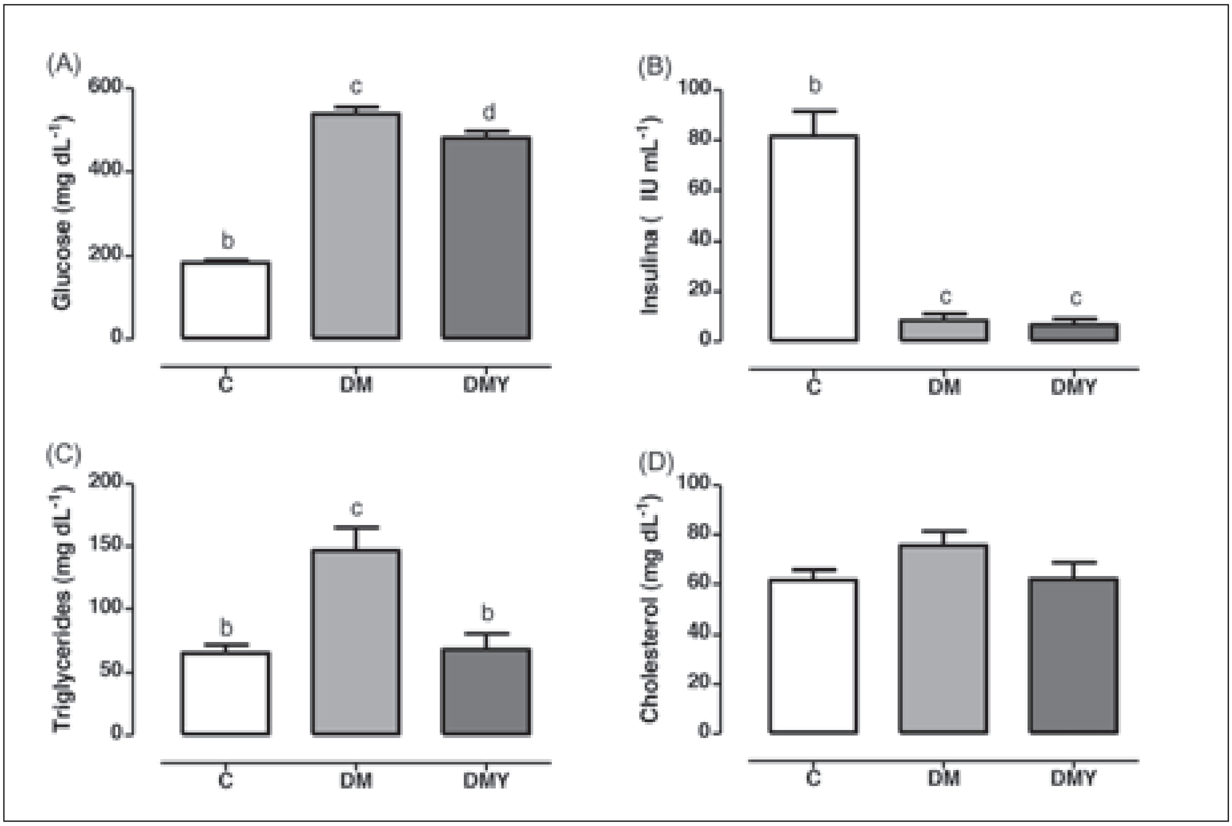

Fig. 3.—Blood glucose level (A), serum insulin (B), triglycerides (C) and cholesterol (D) concentrations, after of the 5 weeks of experiment, respectively. C, control group (n=6); DM, diabetic group fed with high-fat diet (n=8); DMY, diabetic group fed with high-fat diet containing yam flour (n=8). Mean values with different superscripts (a, b and c) are significantly different. One-way ANOVA, P < 0.05.

Discussion

Treatment regimen for diabetes mellitus has been aimed at ensuring weight control.[21] In this context, insulin deprivation (type 1 diabetes) results in an adipose tissue loss. The restore of insulin results in lipogenesis and maintenance of adipose tissue.[22,23] The STZ-induced diabetic female rats showed lower gonadal fat mass which could be associated with absence of adequate amounts of insulin.[7] However, the Brazilian yam flour was associated with an abbreviation of gonadal fat loss. The yams (*Dioscorea* sp.) are abundant in steroidal saponins, compound with insulin-like properties. The saponins stimulate glucose uptake enhancing Glut4 expression, contributing to storage of glucose as glycogen in adipocytes.[21] Although the present study has not assessed glucose uptake, these pathways help to explain the gonadal fat mass observed after treatment with *Dioscorea bulbifera*.

Altered fatty acids metabolism in adipose tissue could lead to elevated circulating fatty acid levels and thereby influence the circulating levels of triglycerides.[24] In patients with insulin deprivation (type 1 diabetes), the adipocytes may uncouple the adipose tissue from circulating fatty acids so that fatty acids are instead shunted to the liver, resulting increased synthesis of triglycerides.[24,25] In the present study, diabetic rat of DM group developed hypertriglyceridemia, however the DMY group showed maintenance of liver and serum triglycerides. Chen et al.[16] observed in mice with diet, containing 25% of *Dioscorea japonica* Thunb var. *pseudojaponica* Yamamoto, which plasma and hepatic triglycerides tended to decrease. These results were associated to yam fiber, which decreased the absorptions of triglycerides. Subsequently, Boban et al.[26] reported a reduction in serum and liver triglycerides in rats with mucilages isolated from *Dioscorea esculenta*. The authors suggested that mucilages contribute to a decrease in the synthesis and secretion of triglycerides by hepatocytes. In previous study[7] we observed that the small intestine was histomorphologically remodeled. This contributed to abbreviation of hyperglycaemia and to control of liver and serum triglycerides.

Type 1 diabetic individuals have been proposed to have reduced synthesis and elevated absorption of cholesterol.[27] And many of these individuals have a Western diet containing a lot of fat from animal origins.[28] The diet used in this model contain lard, however the DMY showed serum cholesterol 17% lower in regard to untreated DM group. Elekofehinti et al.[21] related hypocholesterolemic effects of saponin, inhibiting cholesterol and/or bile acid absorption. Probably, the Brazilian yam (*Dioscorea bulbifera*) may be associated with a decreased intestinal absorption of cholesterol.

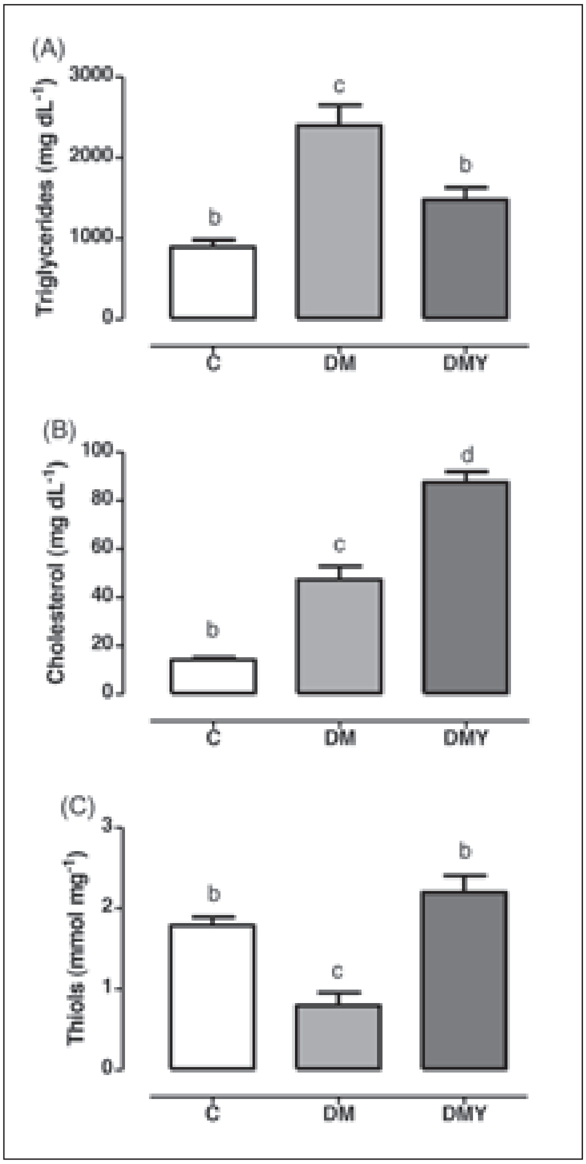

Fig. 4.—Liver triglycerides (A), cholesterol (B) and thiols (C) concentrations, after of the 5 weeks of experiment, respectively. C, control group (n=6); DM, diabetic group fed with high-fat diet (n=8); DMY, diabetic group fed with high-fat diet containing yam flour (n=8). Mean values with different superscripts (a, b and c) are significantly different. One-way ANOVA, $P < 0.05$.

However, as observed in rats treated with *Dioscorea opposita*,[29] the DMY group showed higher hepatic cholesterol. Although Nishimura et al.[29] describes that increased liver cholesterol concentration may be due to suppressed release of VLDL from the liver, Wang et al.[30] showed that ethanol extract of *Dioscorea bulbifera* can induce liver toxicity and mechanism of such hepatoxicity may be related to liver oxidative stress injury in mice. A limitation of the present study is the fact that we could not evaluate the fat accumulation in hepatocytes by histological method. Even so, the thiols analysis showed a possible protective effect of the Brazilian yam to liver.

In fatty liver disease occurs increase in the free cholesterol resulting in depletion of mitochondrial glutathione concentrations and oxidative stress.[31] Within this framework, Ghosh et al.[32] and Pari et al.[33] provide evidence considering the *Dioscorea bulbifera* and steroid saponin, respectively, as natural antioxidants upregulating antioxidant enzyme activities reducing lipid peroxidation. These findings were corroborated because the components of yam could have contributed for the liver antioxidant defense by maintaining the thiol activity.

The Brazilian yam (*Dioscorea bulbifera*) alleviated the consequences of the experimental diabetic disease, suggesting protection to hypertriglyceridemia and lipid peroxidation.

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