

Vochysia rufa and glibenclamide promote effects on oxidative damage and in proteins implicated in vesicular trafficking in diabetic rat brain

Abstract

Many Vochysiaceae species are widely used in folk medicine to treat some diseases. *Vochysia rufa*, popularly known as “quina-doce”, has been used in folk medicine to treat type 1 and type 2 diabetes mellitus in the state of Minas Gerais, Brazil. Although the antidiabetic and antioxidant effects and phytochemical profile of *Vochysia rufa* have already been elucidated, further studies are needed on the effects of this treatment in specific tissues, such as the brain. This study investigated the effect of aqueous extract of *Vochysia rufa* in diabetic rat brains and for this purpose, oxidative stress markers and the expression/localization of proteins implicated in vesicular trafficking were evaluated. Thirty-two rats were randomized into four groups (non-diabetic, diabetic non-treated, diabetic treated for 43 days with glibenclamide - 6 mg/kg or *Vochysia rufa* - 500 mg/kg). The extract was administered by gavage for 43 days. Analyses were conducted of enzymes concentration and activity in the brain. The protein levels and localization of myosin-Va, CaMKII, synapsin, SNAP-25 and GLUT4 were also analyzed. *Vochysia rufa* extract decreased superoxide dismutase, glutathione peroxidase, reduced glutathione, total sulfhydryl and lipid peroxidation levels and increased glutathione S-transferase levels. Additionally, *Vochysia rufa* treatment increased the expression of myosin-Va, CaMKII and also synapsin, which were confirmed by immunolocalization. The treatment with aqueous extract of *Vochysia rufa* reduces oxidative stress on diabetic rat and protecting the brain from damage caused by hyperglycemia.

Keywords: diabetes mellitus, oxidative stress, myosin-Va, synapsin, CaMKII

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Introduction

Diabetes is characterized by persistent hyperglycemia, which leads to increasing reactive oxygen species and consequent changes tissue damage.¹⁻³ There is an increasing appreciation that memory and learning impairment,^{4,5} as well as morphological changes, such as decreased nuclear diameter of pyramidal neurons of the hippocampus is related with diabetes.⁶ Hyperglycemia leads to imbalance in the oxidative status of the neural tissue, increasing free radical production via auto-oxidation of glucose, glycation of proteins and low production of antioxidant defenses, favoring an oxidative stress situation,¹ could be controlled by antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase.⁷ Hyperglycemia alters cellular redox balance and protein trafficking involved in synaptic vesicle docking. This may be related due to changes in expression of myosins,^{6,8,9} GLUT4,¹⁰ pre synaptic proteins, such as synapsin-1, synaptophysin and SNAP-25.¹¹ Decreased CaMKII phosphorylation in the hippocampus, which may be associated with cognitive dysfunction, is also observed.¹² Many plants have confirmed hypoglycemic properties¹³ and several studies appointed that plant extracts are hypoglycemic agents and alleviates oxidative stress in diabetic rats^{14,15} such as *Pouteria ramiflora*,⁶ which reduced the oxidative damage in brain tissue; *Teucrium polium*,¹⁶ ameliorate memory impairments and brain tissue oxidative damage and *Vochysia rufa*, that was able to control glycemic levels and reduce oxidative stress in liver.¹⁷

Thus, the present study was designed to evaluate the effect of *Vochysia rufa* treatment on oxidative stress and expression of proteins implicated in vesicular trafficking such as myosin-Va, CaMKII, synapsin-1, SNAP-25, GLUT4 and the distribution of these proteins in the cortex and hippocampal regions of the diabetic rat brain.

Material and methods

Plant material and aqueous extract

Stem barks from *Vochysia rufa* Mart. were collected from the Cerrado biome in the outskirts of Abadia dos Dourados/MG, Brazil, (latitude 18° 27' 50.5" and longitude 47° 23' 37.2") from October, 2010 to February 2011. The plant was botanically identified and a voucher specimen deposited (number 58,888) at the Herbarium Uberlandensis of the Biology Institute at Federal University of Uberlândia, Uberlândia/MG, Brazil. Then, using a stove at 40°C, the stem barks were dried and powdered. Through maceration of 200 g of powdered bark in 1 L of distilled water for 24 h (1:5 w/v) at room temperature, the aqueous extract was obtained. The extract was filtered and centrifuged at 2000 xg at 4°C, for 15 min. Thus, the supernatant was collected, frozen, lyophilized and stored at -20°C. The phytochemical characterization of the *Vochysia rufa* extract and its molecular identification by HPLC/ESI-MS/MS were revealed by Gouveia et al.¹⁸ and Franco et al.¹⁹ This study received authorization for access and remittance of genetic material for scientific research by SISGEN (n° A933BED).

Animals

Male Wistar rats (approximately 8 weeks and 200-220 g) were housed under standard conditions (22 ± 1 °C, humidity 60 ± 5 %, and 12 h light/12 h dark cycle) with food (65.82% carbohydrate, 5.36% fiber, 21.0% protein and 4.96% fat; Bio base, Brazil) and water *ad libitum*. Procedures for animal handling and use followed the resolutions proposed by the Brazilian Society of Laboratory Animal Science and the Ethics Committee for Animal Research of the Federal University of Uberlândia, Uberlândia/MG, Brazil (CEUA/UFU 060/10).

Induction of diabetes mellitus

The rats were fasted for 24 h, anesthetized by intraperitoneal injection of xylazine (10 mg/kg body weight, bw)/ketamine (75 mg/kg bw) and streptozotocin (40 mg/kg bw, 0.01 M citrate buffer, pH 4.5; Sigma-Aldrich, USA) via the penile vein (2 mL/kg bw) was injected.^{6,8,9,20,21} Non-diabetic rats received equal volume of citrate buffer through penile vein. Then animals were fasted for another 90 min after injection. Ten days after the streptozotocin injection, rats with fasting blood glucose levels above 250 mg/dL were scored as diabetic. Glucose levels were monitored throughout the experiment Biocheck Strips Glucose Test (Bioeasy, Brazil).

Groups and treatments

The rats were randomly divided into four groups (n=8 rats/group): non-diabetic (ND), diabetic (D), diabetic treated with glibenclamide (6 mg/kg bw orally; DG; Biosintética Farmacêutica Ltda, Brazil), and diabetic treated with *Vochysia rufa* extract (500 mg/kg bw orally; DV). The rats received water and treatment administered by gavage for 43 days. Body weight was recorded weekly. After this period, the animals were fasted for 12 h, anesthetized with xylazine (10 mg/kg body weight, bw)/ketamine (75 mg/kg bw), followed by cervical dislocation. Afterwards, brains were surgically removed and immersed in liquid nitrogen (n=4 rats/group) or 10% formaldehyde solution (n = 4 rats/group).

Biochemical analysis

Each brain was homogenized separately on ice in homogenization buffer (50 mM Tris-HCl, pH 7.5, 10 mM EDTA, 2 mM EGTA, 2 mM dithiothreitol, 1 mM benzamidine, 0.5 mM phenylmethanesulfonyl-fluoride, 0.1 M aprotinin, 0.1 mM pepabloc). The homogenates were centrifuged at $15,000 \times g$ for 30 min at 4 °C, and the total protein concentration in the supernatants was measured by the Bradford assay.²²

Oxidative stress biomarkers

Superoxide dismutase (SOD) activity was determined by adenocromo inhibition during adrenaline oxidation. The results were expressed as U/ μ g of protein.²³ Glutathione peroxidase (GPx) activity was quantified by the oxidation of NADPH in the presence of glutathione reductase (GR), measured at 340 nm and expressed in $\text{mmol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$.²⁴ Glutathione S-transferase (GST) activity was measured at 340 nm using CDNB as substrate and a 0.15 M reduced glutathione (GSH).²⁵ The levels of GSH were determined using DTNB (2-dithionitrobenzoic acid)²⁶ and the level of sulfhydryl was determined using 5,5-dithiobis (2-nitrobenzoic acid) (DTNB), according to the method of Faure and Lafond.²⁷ The results were expressed in $\mu\text{mol}/\text{mg}$ of protein. Also, the level of malonyldialdehyde (MDA), as a substance that reacts with thiobarbituric acid were estimated colorimetrically.²⁸ The results were expressed in $\text{nmol} \cdot \text{g}^{-1}$ tissue.

Antibodies

Rabbit affinity-purified polyclonal antibodies included: anti-chicken brain myosin-Va (anti-myosin-Va) head domain recombinant protein²⁹⁻³¹ and anti-glucose transporter GLUT4 (anti-GLUT4) by Abcam Inc. (Cambridge, USA). Mouse monoclonal antibodies used: anti-calcium/calmodulin-dependent protein kinase II α (anti- αCaMKII), anti-synaptosomal-associated protein-25 (anti-SNAP-25), and anti-synapsin by BD Transduction (San Jose, USA) and anti- β -actin by Sigma-Aldrich (St. Louis, USA). Secondary antibodies (anti-mouse IgG and anti-rabbit IgG) conjugated to peroxidase were obtained from GE Healthcare (Buckinghamshire, UK).

Western blotting

Aliquots of supernatant samples (see Biochemical analysis) containing 30 μg of protein were solubilized in a small volume of electrophoresis sample buffer,³² analyzed by SDS-PAGE on 5-22% polyacrylamide gradients, and electroblotted onto nitrocellulose membranes in Tris-glycine buffer. The membranes were probed with 0.2 $\mu\text{g}/\text{mL}$ primary antibodies. β -actin antibody was used as a control. The membranes were incubated with peroxidase-conjugated anti-rabbit IgG and anti-mouse IgG diluted 1:2,000. Antibodies bound to the membranes were visualized by chemiluminescence after incubation with ECLTM followed by exposure to HyperfilmTM (GE Healthcare, Sweden), according to the manufacture's instructions. The intensity of the protein bands was analyzed and compared using Scion Image software, version Alpha 4.0.3.2 (Scion Corporation, USA), and the results were expressed as percentage of total protein content.

Immunohistochemistry

The brains were fixed with 10% formaldehyde solution in 0.1 M phosphate-buffered saline (pH 7.4) for 24 h, dehydrated in ethanol, rinsed in xylene and embedded in paraffin.^{6,9,21} Seven-micrometer sections were pretreated in microwave for 5 min with 4 mM citrate buffer (pH 6.0) containing 0.025% Tween 20. The sections were then incubated with primary antibodies for 16 h followed by incubation with a Post Primary Block NovoLinkTM Max Polymer Detection System (Novocastra Laboratories Ltd., UK). The sections were incubated with the NovoLink polymer for 30 min at 37 °C. Chromogen was developed using 3,3'-diaminobenzidine, and then the material was counterstained with hematoxylin, dehydrated, mounted with ERV-Mount (EasyPath, Brazil) and analyzed with the 40 \times objective lens of Leica DM500 microscope (Microsystems, Germany). Negative controls were prepared by omitting the primary antibody from the reaction.

Statistical analysis

All values obtained were expressed as mean \pm SEM (standard error mean). Statistical analysis was performed with the Student t-test, and data were analyzed using SigmaStat 3.5 software (Systat Software Inc., USA). A p-value < 0.05 was considered significant.

Results and discussion

It has been reported that deleterious effects of reactive oxygen species (ROS)-mediated injury are frequently observed in diabetic patients and animal models.⁴ The deleterious effects are related to cellular proteins disruption, lipid membranes oxidation, nucleic acids damage, and cell death.¹ The central nervous system is especially vulnerable to oxidative damage.³³ In the present study, oxidative stress markers and antioxidant enzyme levels were evaluated in diabetic rats treated with glibenclamide and an aqueous extract of *Vochysia rufa*. Figure 1 depicts SOD, GPx and GST enzymes activities, GSH enzyme concentration, total sulfhydryl and MDA in the brain. GPx and GST activities decreased in the diabetic when compared to non-diabetic rats, meanwhile SOD, GSH, total sulfhydryl and MDA levels increased. These results are attributable to a high rate of oxygen consumption and high polyunsaturated fatty acid content especially pronounced in diabetic brains,³⁴ once hyperglycemia status reduces antioxidant levels and increases free radicals production with subsequent activation of redox-sensitive genes.^{35,36}

It is believed that plants with antioxidant properties can prevent or protect tissues against the injuries of free radicals.³⁷ Treatment with *Vochysia rufa* aqueous extract significantly decreased SOD, GSH and GPx activities, total sulfhydryl and MDA levels. Moreover, the

group treated with the plant extract ameliorated GST activity. The gender *Vochysia* has abundant polyphenols and triterpenes³⁸ with antidiabetic properties,³⁹ but the total polyphenols, flavonoids and condensed tannins content were only recently revealed for *Vochysia rufa* ethanolic extract of stem bark.¹⁹

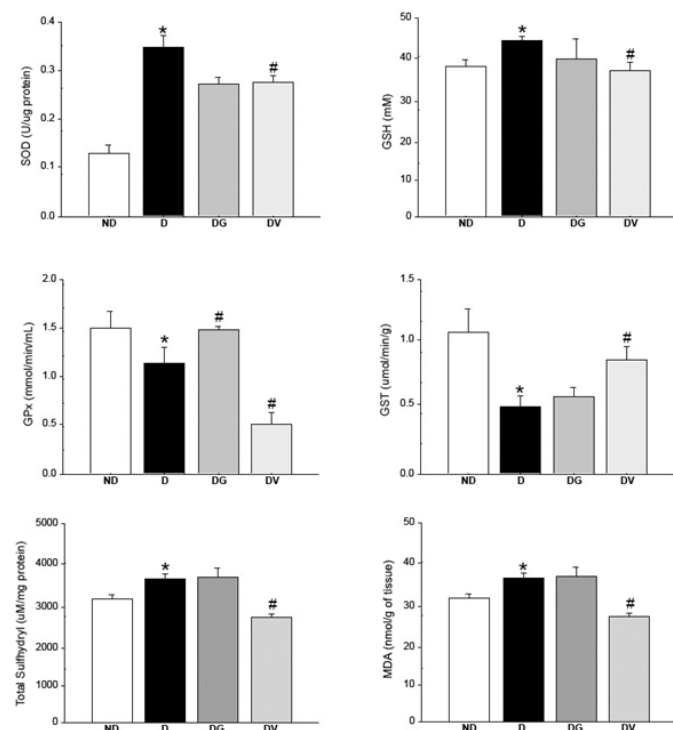


Figure 1 Effect of treatment with *Vochysia rufa* extract on superoxide dismutase (SOD), reduced glutathione (GSH), glutathione peroxidase (GPx) and glutathione S-transferase (GST) activity, total sulfhydryl and malondialdehyde (MDA) concentrations in the brain of streptozotocin-induced diabetic Wistar rats. ND: non-diabetic, D: diabetic, DG: diabetic with glibenclamide, DV: diabetic with *Vochysia rufa*. Data are reported as mean \pm SEM (n = 4 rats/group). *compared to non-diabetic, # compared to diabetic.

Moraes et al.¹⁷ and Gouveia et al.¹⁸ using the same aqueous extract of the present study concluded that hepatic and pancreatic oxidative stress, respectively, caused by streptozotocin-induced diabetes, reduced after treatment. Moreover, Gouveia et al.⁴⁰ revealed partial composition of *Vochysia rufa* stem bark and its effect against oxidative damage in endothelial cells, and in the *Vochysia rufa* extract was found to be composed of free sugars (inositol, galactose, glucose, mannose, sucrose, arabinose, and ribose) as the main metabolites.³⁹ We observed the same protective effect in the rat brain.

Several studies have shown that plant extracts can reverse changes in the rat brain caused by diabetes. For instance, a curcumin supplement decreases lipid peroxidation levels,⁴¹ *Bacopa monnieri* modulates SOD and GPx activities in different brain regions⁴² and *Pouteria ramiflora* increases GPx and SOD levels and also decreases lipid peroxidation.⁶ On the other hand, when used as a positive control glibenclamide increased GPx and decreased SOD activities, when compared to diabetic groups (Figure 1). This sulfonylurea affects beta cells stimulating insulin secretion,^{43,44} which probably reduces oxidative stress diminishing the hyperglycemic status.

Diabetes is related to changes in neurotransmission that may cause cognitive dysfunction.⁵ Neurotransmission only occurs after formation of a complex, evolving proteins of the synaptic vesicle and the plasma membrane. However, if one side of this pair is missing, the complex will be unstructured.^{45,46} Proteins such as myosin-Va, CaMKII,

synapsin and SNAP-25 are involved in neurotransmission, whereas GLUT4 participates as an insulin-sensitive glucose transporter. Figure 2 shows the immunoreactivities of myosin-Va (190 kDa), α -CaMKII (52 kDa), synapsin (80 kDa), SNAP-25 (25 kDa) and GLUT4 (56 kDa) measured densitometrically. Distribution of these proteins in the frontal (Figure 3-4) and occipital (Figure 5-6) cortex and hippocampus (Figure 7) was revealed by immunohistochemistry.

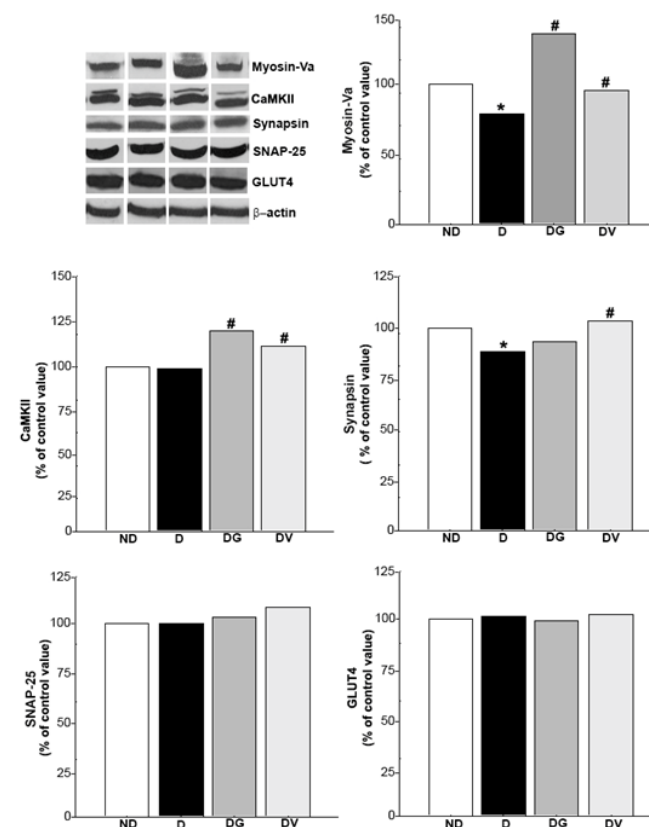


Figure 2 Effect of treatment with *Vochysia rufa* extract on myosin-Va, α -CaMKII, synapsin, SNAP-25 and GLUT4 protein levels in the brain of streptozotocin-induced diabetic Wistar rats. ND: non-diabetic, D: diabetic, DG: diabetic with glibenclamide, DV: diabetic with *Vochysia rufa*. The protein level in the immunoblot was determined by densitometric readings and data are expressed as percentage within groups (n = 4 rats/group). *compared to non-diabetic, # compared to diabetic.

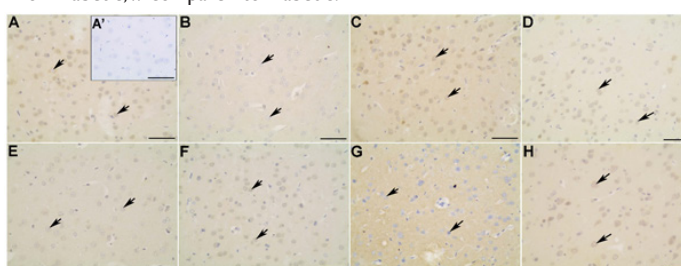


Figure 3 Myosin-Va (A-D) and α -CaMKII (E-H) distribution in the frontal cortex of non-diabetic (A; A'), diabetic (B; F), diabetic treated with glibenclamide (C; G), and diabetic treated with *Vochysia rufa* extract (D; H) rat brains. Pyramidal neurons (arrow). Negative control (A'). Bar: 25 μ m (A-H) and 50 μ m (A').

Western blot analysis displayed that diabetic rats had decreased levels of myosin-Va in the brain when compared to the non-diabetic group. Low levels of this protein was also observed in diabetic rats by da Costa et al.,^{6,9} but treatment with either *Vochysia rufa* or glibenclamide reversed this effect (Figure 2). Glibenclamide also

reverse damage caused by diabetes on myosin-IIB expression and reduce the patho-physiological changes in the brain.²⁰ Myosin-Va distribution in non-diabetic is more intense in the cytoplasmic soma and extension of pyramidal neurons with intense perinuclear labeling in both cortex (Figure 3A-D; Figure 5A-D), a similar pattern was described by Takagishi et al.⁴⁷. Myosin-Va is a molecular motor associated with transport vesicles in neurons⁴⁸ and its expression changed after treatment with aqueous *Pouteria ramiflora* extract, likely as a response to the plant's antioxidant and neuroprotective properties.⁶ Similarly, it is possible that the antioxidant properties of the *Vochysia* genus had the same effect.⁴⁹

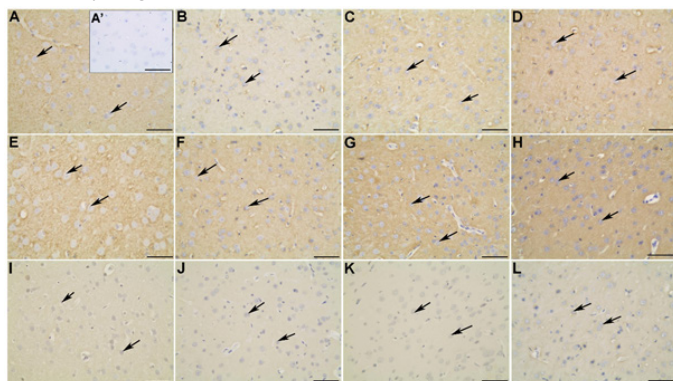


Figure 4 Synapsin-I (A-D), SNAP-25 (E-H) and GLUT4 (I-L) distribution in the frontal cortex of non-diabetic (A; A'; E; I), diabetic (B; F; J), diabetic treated with glibenclamide (C; G; K), and diabetic treated with *Vochysia rufa* extract (D; H; L) rat brains. Pyramidal neurons (arrow). Negative control (A'). Bar: 25 µm (A-L) and 50 µm (A').

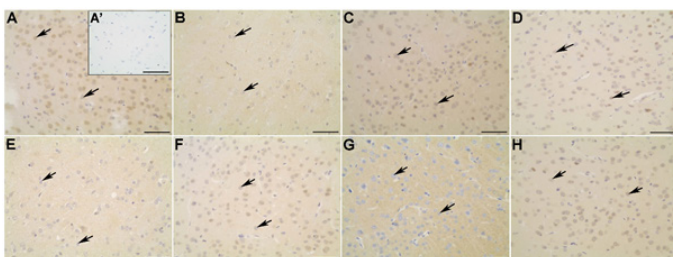


Figure 5 Myosin-Va (A-D) and α -CaMKII (E-H) distribution in the occipital cortex of non-diabetic (A; A'; E), diabetic (B; F), diabetic treated with glibenclamide (C; G), and diabetic treated with *Vochysia rufa* extract (D; H) rat brains. Pyramidal neurons (arrow). Negative control (A'). Bar: 25 µm (A-H), and 50 µm (A').

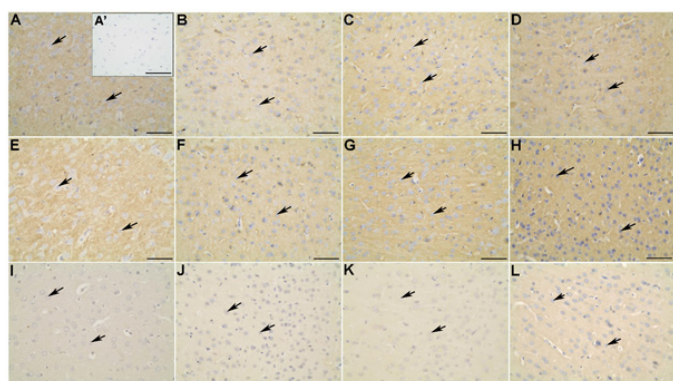


Figure 6 Synapsin-I (A-D), SNAP 25 (E-H) and GLUT4 (I-L) distribution in the occipital cortex of the non-diabetic (A; A'; E; I), diabetic (B; F; J), diabetic treated with glibenclamide (C; G; K), and diabetic treated with *Vochysia rufa* extract (D; H; L) rat brains. Pyramidal neurons (arrow). Negative control (A'). Bar: 25 µm (A-L), and 50 µm (A').

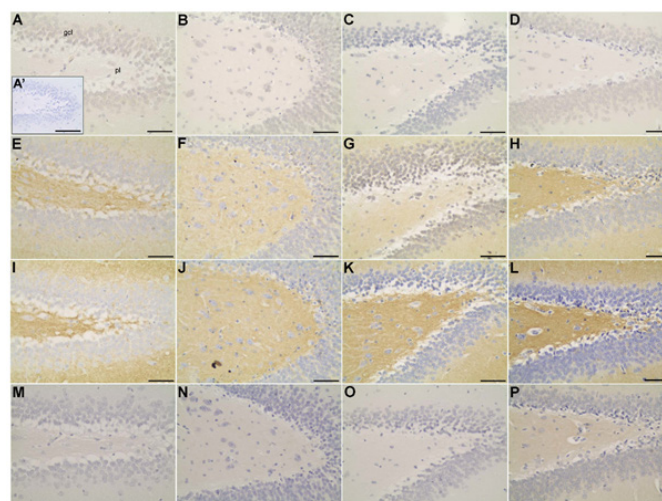


Figure 7 Immunolocalization of α -CaMKII (A-D), synapsin-I (E-H), SNAP 25 (I-L) and GLUT4 (M-P) in dentate gyrus of the hippocampus of non-diabetic (A; A'; E; I; M), diabetic (B; F; J; N), diabetic treated with glibenclamide (C; G; K; O), and diabetic treated with *Vochysia rufa* extract (D; H; L; P) rat brains. Neurons of the granular layer (gcl); neurons of the polymorphic layer (pl). Negative control (A'). Bar: 25 µm (A-P), and 50 µm (A').

Protein levels of α -CaMKII were kept the same, comparing non-diabetic and diabetic rats, but increased when treated with glibenclamide or *Vochysia rufa* extract (Figure 2). α -CaMKII distribution was uniform in non-diabetic and diabetic rat brains, and produced less intense marking in the cytoplasm soma and perinuclear region in frontal cortex (Figure 3 E-H). The nucleus of neurons were stained more in rats treated with glibenclamide than in non-diabetic, diabetic and diabetic treated with *Vochysia rufa* extract in occipital cortex (Figure 5E-H). CaMKII is an important enzyme in the regulation of glutamatergic synapses⁵⁰ and is essential for synthesis of insulin granules, modulation of cytoplasmic content of ATP, and activation of synapsin.⁵¹ Thus, it is possible that hyperglycemia activates CaMKII and alter phosphorylation of its multiple targets to exert both acute and chronic effects in many tissues,⁵² and treatment with plant extract or antidiabetic stimulate insulin release by CaMKII action.

Although streptozotocin-induced diabetes cause prominent changes in neuronal calcium homeostasis⁵³ as well as an increase CaMKII of total amount, was observed in central nervous system and in different tissues of diabetes types 1 and 2.^{12,54} Those alterations were not observed in our study, and the activation mechanism for this disease has not been elucidated yet. Other important point is that the oxidative stress potentiates synaptic transmission via activation of CaMKII in several pathophysiological conditions,^{55,56} including diabetes. Synapsin I is a substrate for CaMKII in the pancreas, but this event appears not to be important for the mobilization of insulin granules.⁵⁷ Diabetic rats have decreased synapsin in the brain when compared to the non-diabetic group, whereas treatment with *Vochysia rufa* extract increased the protein levels (Figure 2). Plants are constantly used to improve hyperglycemia and thereby oxidative stress.^{15,58,59}

Moreover, synapsin distribution in the frontal (Figure 4A-D) and occipital (Figure 6A-D) cortex of non-diabetic rat brains was intense in the soma and extension of pyramidal neurons compared to diabetic, and in the cytoplasm of neurons of the granular layer and polymorphic layer in hippocampus (Figure 7E-H). Immunohistochemical staining demonstrated an intense immunolabeling of synapsin I in hippocampal

pyramidal neurons in type 1 diabetic rats.¹² This protein is involved in the regulation of exocytotic release vesicles.⁶⁰ Our blotting analyses showed no differences in SNAP-25 and GLUT4 expression between groups (Figure 2) even in brain distribution. SNAP-25 participates in SNARE complex assembly^{61,62} and the glucose pulse paradigm induced its expression.⁶³ SNAP-25 was intense immunolabeling in the frontal (Figure 4E-H) and occipital (Figure 6E-H) cortex of the non-diabetics and diabetics, predominantly in the soma and extension of pyramidal neurons, and in the cytoplasm of neurons of the granular layer and polymorphic layer in hippocampus (Figure 7I-L). GLUT4 distribution in the frontal (Figure 4 I-L) and occipital (Figure 6 I-L) cortex does not differ among the groups, with weak and diffuse label in the cytoplasm and fibers, including hippocampus (Figure 7 M-P). There is no difference in the expression of *glut4* mRNA in the frontal cortex⁶⁴ and expression of GLUT4 protein in the cerebral cortex of streptozotocin-diabetic rats¹⁰ even with increased glucose levels.

Conclusion

Oxidative stress associated with the expression of protein vesicles that may affect synaptic function and plasticity or alter the release of vesicles, appears to be a presynaptic deleterious mode of action of reactive oxygen species. We suggest that, by decreasing oxidative stress, *Vochysia rufa* extract and glibenclamide improved the proteins levels associated with vesicle trafficking and anchoring. Uncontrolled diabetes markedly alters hippocampal genes expression, particularly for genes thought to be involved in synaptic function, plasticity and for neurogenesis. Plant extracts had no effect on the levels of binding protein for complex formation, but high concentration of polyphenols could decrease the interaction between vesicle proteins. It was confirmed that *Vochysia rufa* extract increase expression levels of the myosin-Va, CaMKII and synapsin, and reduces oxidative stress, thus, protecting the brain from damage caused by hyperglycemia. Future studies are needed to understand the mechanism of action of *Vochysia rufa* in brain of diabetics.

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Author contributions

AVC and LKC performed the acquisition and interpretation of data, and were involved in drafting the manuscript. AAAR was involved in the interpretation of data and critical revision of the manuscript for important intellectual content. FSE conceived and designed the study. All authors reviewed and approved of the final manuscript.

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Competing interests

The authors have declared that no competing interests exist.

References

- Maritim AC, Sanders RA, Watkin JB. Diabetes, oxidative stress, and antioxidants: a review. *J Biochem Mol Toxicol*. 2003;17(1):24–38.
- Ugochukwu NH, Cobourne MK. Modification of renal oxidative stress and lipid peroxidation in streptozotocin-induced diabetic rats treated

with extracts from *Gongronema latifolium* leaves. *Clin Chim Acta*. 2003;336(1–2):73–81.

- Forbes JM, Cooper ME. Mechanisms of diabetic complications. *Physiol Rev*. 2013;93(1):137–188.
- Biessels GJ, Kappelle AC, Bravenboer B, et al. Cerebral function in diabetes mellitus. *Diabetologia*. 1994;37(7):643–650.
- McCrimmon RJ, Ryan CM, Frier BM. Diabetes and cognitive dysfunction. *Lancet*. 2012;379(9833):2291–2299.
- Costa AV, Cal bria LK, Furtado FB, et al. Neuroprotective effects of *Pouteria ramiflora* (Mart.) Radlk (Sapotaceae) extract on the brains of rats with streptozotocin-induced diabetes. *Metab Brain Dis*. 2013;28(3):411–419.
- Robertson RP, Harmon J, Tran PO, et al. Beta-cell glucose toxicity, lipotoxicity, and chronic oxidative stress in type 2 diabetes. *Diabetes*. 2004;53(Suppl 1):S119–S124.
- Cal bria LK, da Cruz GC, Nascimento R, et al. Overexpression of myosin-IIB in the brain of a rat model of streptozotocin-induced diabetes. *J Neurol Sci*. 2011;303(1–2):43–49.
- Costa AV, Cal bria LK, Nascimento R, et al. The streptozotocin-induced rat model of diabetes mellitus evidences significant reduction of myosin-Va expression in the brain. *Metab Brain Dis*. 2011;26(4):247–251.
- Vannucci SJ, Koehler Stec EM, Li K, et al. GLUT4 glucose transporter expression in rodent brain: effect of diabetes. *Brain Res*. 1998;797(1):1–11.
- VanGuilder HD, Brucklacher RM, Patel K, et al. Diabetes downregulates presynaptic proteins and reduces basal synapsin I phosphorylation in rat retina. *Eur J Neurosci*. 2008;28(1):1–11.
- Liao MH, Xiang YC, Huang JY, et al. The disturbance of hippocampal CaMKII/PKA/PKC phosphorylation in early experimental diabetes mellitus. *CNS Neurosci Ther*. 2013;19(5):329–336.
- Negri G. Diabetes melito: plantas e princ pios ativos naturais hipoglicemiantes. *Rev Bras Cien Farm*. 2005;41(2):121–142.
- Taleb Senouci D, Ghomari H, Krouf D, et al. Antioxidant effect of *Ajuga iva* aqueous extract in streptozotocin-induced diabetic rats. *Phytomedicine*. 2009;16(6–7):623–631.
- Chang CL, Lin Y, Bartolome AP, et al. Herbal therapies for type 2 diabetes mellitus: chemistry, biology, and potential application of selected plants and compounds. *Evid Based Complement Alternat Med*. 2013;2013:1–33.
- Mousavi SM, Niazmand S, Hosseini M, et al. Beneficial effects of *Teucrium polium* and metformin on diabetes-induced memory impairments and brain tissue oxidative damage in rats. *Int J Alzheimers Dis*. 2015;2015:493729.
- Moraes IB, Manzan Martins C, de Gouveia NM, et al. Polyploidy analysis and attenuation of oxidative stress in hepatic tissue of STZ-induced diabetic rats treated with an aqueous extract of *Vochysia rufa*. *Evid Based Complement Alternat Med*. 2015;2015:1–8.
- Gouveia NM, Rodrigues WF, Sousa RMF, et al. Phytochemical characterization of the *Vochysia rufa* (Vochysiaceae) extract and its effects on oxidative stress in the pancreata of streptozotocin-induced diabetic rats. *PLoS One*. 2017;12(10):e0184807.
- Franco RR, Justino AB, Martins MM, et al. Phytoscreening of Vochysiaceae species: Molecular identification by HPLC/ESI-MS/MS and evaluating of their antioxidant activity and inhibitory potential against human α -amylase and protein glycation. *Bioorg Chem*. 2019;91:103122.
- Costa AV, Cal bria LK, de Souza Santos P, et al. Glibenclamide treatment modulates the expression and localization of myosin-IIB in diabetic rat brain. *J Neurol Sci*. 2014;340(1–2):159–164.

21. Calábria LK, Vieira da Costa A, da Silva Oliveira RJ, et al. Myosins are differentially expressed under oxidative stress in chronic streptozotocin-induced diabetic rat brains. *ISRN Neurosci*. 2013;2013:423931.
22. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*. 1976;72:248–254.
23. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem*. 1972;247(10):3170–3175.
24. Flohe L, Gunzler W. Assays of glutathione peroxidase. *Methods Enzymol*. 1984;105:114–121.
25. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *J Biol Chem*. 1974;249(22):7130–7139.
26. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. *J Lab Clin Med*. 1963;61:882–888.
27. Faure P, Lafond JL. Measurement of plasma sulfhydryl and carbonyl groups as a possible indicator of protein oxidation. In: Favier A, Cadet J, Kalyanaraman B, Fontecave M, Pierre JL, editors. *Analysis of free radicals in biological systems*. Birkhäuser Verlag: Basel; 1995.(p.237–248).
28. Hermes Lima M, Willmore WG, Storey KB. Quantification of lipid peroxidation in tissue extracts based on Fe(III)xylenol orange complex formation. *Free Radic Biol Med*. 1995;19(3):271–280.
29. Espreafico EM, Cheney RE, Matteoli M, et al. Primary structure and cellular localization of chicken brain myosin-V (p190), an unconventional myosin with calmodulin light chains. *J Cell Biol*. 1992;119(6):1541–1557.
30. Suter DM, Espindola FS, Lin CH, et al. Localization of unconventional myosins V and VI in neuronal growth cones. *J Neurobiol*. 2000;42(3):370–382.
31. Espindola FS, Banzi SR, Calábria LK, et al. Localization of myosin-Va in subpopulations of cells in rat endocrine organs. *Cell Tissue Res*. 2008;333(2):263–279.
32. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA*. 1979;76(9):4350–4354.
33. Barcia JM, Flores-Bellver M, Muriach M, et al. Matching Diabetes and Alcoholism: oxidative stress, inflammation, and neurogenesis are commonly involved. *Mediators Inflamm*. 2015;2015:624287.
34. Bonnefont-Rousselot D. Glucose and reactive oxygen species. *Curr Opin Clin Nutr Metab Care*. 2002;5:561–568.
35. Valko M, Leibfriz D, Moncol J, et al. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol*. 2007;39(1):44–84.
36. Muriach M, Flores-Bellver M, Romero FJ, et al. Diabetes and the brain: oxidative stress, inflammation, and autophagy. *Oxid Med Cell Longev*. 2014;2014:102158.
37. Yazdanparast R, Ardestani A, Jamshidi S. Experimental diabetes treated with *Achillea santolina*: effect on pancreatic oxidative parameters. *J Ethnopharmacol*. 2007;112(1):13–18.
38. Zucaro YL, Compagnone RS, Hess SC, et al. 6 β -hydroxymaslinic acid, a triterpene from *Vochysia ferruginea*. *J Braz Chem Soc*. 2000;11(3):241–244.
39. Sabu MC, Smitha K, Kuttan R. Anti-diabetic activity of green tea polyphenols and their role in reducing oxidative stress in experimental diabetes. *J Ethnopharmacol*. 2002;83(1-2):109–116.
40. Gouveia NM, Ramos S, Martín MA, et al. *Vochysia rufa* stem bark extract protects endothelial cells against high glucose damage. *Medicines (Basel)*. 2017;4(1):1–11.
41. Belviranlı M, Okudan N, Atalık KE, et al. Curcumin improves spatial memory and decreases oxidative damage in aged female rats. *Bio gerontology*. 2013;14(2):187–196.
42. Kapoor R, Srivastava S, Kakkar P. *Bacopa monnieri* modulates antioxidant responses in brain and kidney of diabetic rats. *Environ Toxicol Pharmacol*. 2009;27(1):62–69.
43. Serrano-Martin X, Payares G, Mendoza-León A. Glibenclamide, a blocker of K+(ATP) channels, shows antileishmanial activity in experimental murine cutaneous leishmaniasis. *Antimicrob Agents Chemother*. 2006;50(12):4214–4216.
44. Sokolovska J, Isajevs S, Sugoka O, et al. Comparison of the effects of glibenclamide on metabolic parameters, GLUT1 expression, and liver injury in rats with severe and mild streptozotocin-induced diabetes mellitus. *Medicina (Kaunas)*. 2012;48(10):532–543.
45. Jahn R, Sudhof TC. Membrane fusion and exocytosis. *Annu Rev Biochem*. 1999;68:863–911.
46. Lin RC, Scheller RH. Mechanisms of synaptic vesicle exocytosis. *Annu Rev Cell Dev Biol*. 2000;16:19–49.
47. Takagishi Y, Hashimoto K, Kayahara T, et al. Diminished climbing fiber innervation of Purkinje cells in the cerebellum of myosin Va mutant mice and rats. *Dev Neurobiol*. 2007;67(7):909–923.
48. Hirokawa N, Niwa S, Tanaka Y. Molecular motors in neurons: transport mechanisms and roles in brain function, development, and disease. *Neuron*. 2010;68(4):610–638.
49. Gomes RC, Bonamin F, Darin DD, et al. Antioxidative action of methanolic extract and buthanolic fraction of *Vochysia tucanorum* Mart. in the gastroprotection. *J Ethnopharmacol*. 2009;121(3):466–471.
50. Lisman J, Schulman H, Cline H. The molecular basis of CaMKII function in synaptic and behavioral memory. *Nat Rev Neurosci*. 2002;3(3):175–190.
51. Easom RA. CaM kinase II: a protein kinase with extraordinary talents germane to insulin exocytosis. *Diabetes*. 1999;48(4):675–684.
52. Erickson JR, Pereira L, Wang L, et al. Diabetic hyperglycaemia activates CaMKII and arrhythmias by O-linked glycosylation. *Nature*. 2013;502(7471):372–376.
53. Voitenko NV, Kostyuk EP, Kruglikov IA, et al. Changes in calcium signalling in dorsal horn neurons in rats with streptozotocin-induced diabetes. *Neuroscience*. 1999;94(3):887–890.
54. Bhardwaj SK, Kaur G. Effect of diabetes on calcium/calmodulin dependent protein kinase-II from rat brain. *Neurochem Int*. 1999;35(4):329–335.
55. Palomeque J, Rueda OV, Sapia L, et al. Angiotensin II-induced oxidative stress resets the Ca2+ dependence of Ca2+-calmodulin protein kinase II and promotes a death pathway conserved across different species. *Circ Res*. 2009;105(12):1204–1212.
56. Wagner S, Ruff HM, Weber SL, et al. Reactive oxygen species-activated Ca/calmodulin kinase II δ is required for late I(Na) augmentation leading to cellular Na and Ca overload. *Circ Res*. 2011;108(5):555–565.
57. Krueger KA, Ings EI, Brun AM, et al. Site-specific phosphorylation of synapsin I by Ca2+/calmodulin-dependent protein kinase II in pancreatic betaTC3 cells: synapsin I is not associated with insulin secretory granules. *Diabetes*. 1999;48(3):499–506.
58. Pari L, Latha M. Protective role of *Scoparia dulcis* plant extract on brain antioxidant status and lipidperoxidation in STZ diabetic male Wistar rats. *BMC Complement Altern Med*. 2004;4:4–16.
59. Panickar KS. Beneficial effects of herbs, spices and medicinal plants on the metabolic syndrome, brain and cognitive function. *Cent Nerv Syst Agents Med Chem*. 2013;13(1):13–29.

60. Ceccaldi PE, Grohovaz F, Benfenati F, et al. Dephosphorylated synapsin I anchors synaptic vesicles to actin cytoskeleton: an analysis by videomicroscopy. *J Cell Biol.* 1995;128(5):905–912.
61. Washbourne P, Cansino V, Mathews JR, et al. Cysteine residues of SNAP-25 are required for SNARE disassembly and exocytosis, but not for membrane targeting. *Biochem J.* 2001;357(3):625–634.
62. Greaves J, Prescott GR, Fukata Y, et al. The hydrophobic cysteine-rich domain of SNAP25 couples with downstream residues to mediate membrane interactions and recognition by DHHC palmitoyl transferases. *Mol Biol Cell.* 2009;20(6):1845–1854.
63. Santos GJ, Ferreira SM, Ortis F, et al. Metabolic memory of β -cells controls insulin secretion and is mediated by CaMKII. *Mol Metab.* 2014;3(4):484–489.
64. Soltésová D, Veselá A, Mravec B, et al. Daily profile of glut1 and glut4 expression in tissues inside and outside the blood-brain barrier in control and streptozotocin-treated rats. *Physiol Res.* 2013;62(Suppl 1):S115–S124.