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Effects of strictosamide on mouse brain and kidney Na^+,K^+-ATPase and Mg^{2+}-ATPase activities

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ABSTRACT

Present study reports on the general bioactivity of strictosamide and on its effects on Na^+,K^+-ATPase and Mg^{2+}-ATPase activities of Charles River male mouse. Strictosamide is the main glycoalkaloid of *Sarcocephalus latifolius* (Rubiaceae) leaves and roots, used as medicinal plant in folk medicine. In this work, we studied the *in vitro* effects of various concentrations of strictosamide (0.25, 0.5, 1 or 2 mg/mL) and the *in vivo* effects of single doses (50, 100 or 200 mg/kg, i.p.) of this compound on kidney and brain Na^+,K^+-ATPase and Mg^{2+}-ATPase activities. Results of general study showed that strictosamide is slightly toxic to Charles River mouse (LD_{50} = 723.17 mg/kg), producing CNS depression and kidney toxicity, but the exact mechanism of these effects could not be defined. Strictosamide inhibited the *in vitro* and *in vivo* Mg^{2+}-ATPase activity on kidney but had nonsignificant effect on brain. Furthermore, strictosamide had nonsignificant *in vitro* and *in vivo* effect on kidney Na^+,K^+-ATPase activity but produced an *in vivo* increase of Na^+,K^+-ATPase activity of brain, these findings suggesting that strictosamine may be related to the induction of α_2 isoform of Na^+,K^+-ATPase and may account for the folk use of *Sarcocephalus latifolius* root infusion on hypertension.

1. Introduction

Strictosamide is the (3α,15β,16β,17α)-21-oxo-16-vinil-19,20-dihydro-oxoyoimban-17-il-β-D-glucopyranoside, a glycoalkaloid found in *Sarcocephalus latifolius* (Smith) Bruce (*Nauclea latifolia* Sm.) (Fig. 1).

Sarcocephalus latifolius is a tree member of the Rubiaceae family, growing commonly in some regions of Africa and used for medicinal purposes in folk medicine. The leaves and roots of this plant or its combination in equal proportions have several local use in the treatment of diseases, namely against fever, malaria, gonorrhoea, wounds, coughs, odontalgic problems, stomach aches, disorders of gastrointestinal tract and autonomic system, especially hypertension (Udoh, 1995; Abreu and Pereira, 1998).

Sarcocephalus latifolius exhibit remarkable antimicrobial and antiparasitical activities (Kela et al., 1989; Deeni and Hussain, 1991; Madubunyi, 1995; Benoit-Viscal et al., 1998; Fakae et al., 2000; Tona et al., 2000; Traore-Keita et al., 2000; Abreu and Pereira, 2001; Onyeyili et al., 2001). Studies on mice showed the ethanolic extract of root bark attenuated the hepatoxicity produced by CCl_4 (Madubunyi, 1995). Furthermore, the methanolic extract of leaves potentiated the purinergic neurotransmission by potentiating ATP induced contractions of the bladder, while the root extract depressed purinergic contraction of the bladder by a direct depressant action on the smooth muscle (Udoh, 1995). *In vitro* and *in vivo* studies showed that alkaloid rich extracts of *Sarcocephalus latifolius* could interact with DNA of bacteria and mammalian cells, leading to G2-M cell cycle arrest and heritable DNA damage, as well inducing *in vivo* single-strand breaks in liver, kidney and blood cells (Traore et al., 2000).

Strictosamide isolated from *Sarcocephalus latifolius* was evaluated for its antiplasmodial activity against *Plasmodium falciparum* strains K1 and NF54, exhibiting IC_{50} values of 0.45 and 0.37 μg/mL, respectively (Abreu and Pereira, 2001).

Experiments with *Sarcocephalus latifolius* showed that strictosamide is the most abundant alkaloid isolated from its roots and may be responsible for the slight hipotensive action and the negative inotropic and chronotropic reversible effects produced on frog and rabbit isolated heart (Silva et al., 1964).

Taking into account the folk medicinal use of *Sarcocephalus latifolius* and the reported effects of strictosamide on isolated heart, we have evaluated the behavioural and biological effects of this compound on intact Charles River mouse, in order to investigate the general effects of strictosamide on mice, not yet reported in the literature. Furthermore, we have analysed the *in vitro* and the

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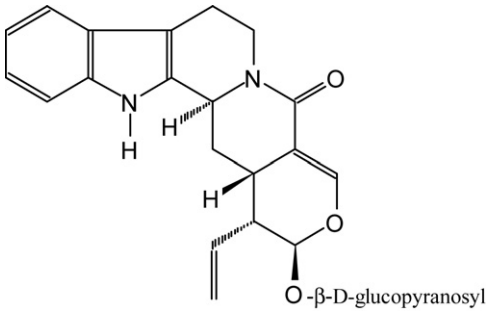

Fig. 1. Strictosamide—the (3α,15β,16β,17α)-21-oxo-16-vinil-19,20-dihydro-oxoyoimban-17-il-β-D glucopyranoside.

in vivo effects of this compound on the Na^+,K^+-ATPase activity of brain and kidney, because the modulation of Na^+,K^+-ATPase activity has been implicated in several pathophysiologic processes, including cardiovascular, renal, neuronal, and metabolic disorders, having in common a dysfunction in salt and water homeostasis (Blanco and Mercer, 1998), and this enzyme is very well expressed in these organs (Gick et al., 1993).

2. Materials and methods

2.1. Drug source and chemicals

Strictosamide was isolated from an ethanolic extract of *Sarcocephalus latifolius* roots, and it was identified by Pedro Abreu of UNL by RMN and MS.

Plant material of *Sarcocephalus latifolius* was collected at Contuboel, Guinea-Bissau, and identified by Dr. E. Martins at the Herbarium of Botany Center of the Institute of Scientific and Tropical Research (Lisboa), where a voucher specimen is deposited.

Column chromatography was performed on Merck silica-gel 60 of 70–230 of mesh, and Merck silica gel plates 0.25 mm were used for analytical TLC.

Ouabain was purchased from Sigma Chemical, Co., St. Louis, MO, USA. BSA and other reagents were purchased from E. Merck, Darmstadt, Germany.

2.2. Strictosamide preparation and identification

Powdered roots of *Sarcocephalus latifolius* (1.5 kg) were extracted in a Soxhlet with ethanol (95%, 4 L), for 48 h, under gentle reflux, yielding a brown residue (140 g), that was fractionated by column chromatography over silica gel using a $CHCl_3$/MeOH step gradient (100:0–20:80). Three fractions of increasing polarity (F1, F2 and F3) were ultimately obtained on combining the eluates on the basis of TLC composition. Column chromatography of F3 (64 g) with $CHCl_3$/MeOH (95:5–80:20) yielded pure strictosamide (25 g). ^{1}H- and ^{13}C NMR spectra were measured on a Brucker ARX-400 at 400 and 100.61 MHz, respectively. MS spectra were obtained by HRCIMS on a Finnigan MAT 95. Data obtained from these measurements were identical to those previously reported by Abreu and Pereira (2001).

2.3. Animals

The experiments were performed with male Charles River mice (weight range 25–30 g) obtained from the Laboratory of Toxicology of University of Évora, Portugal. The animals were housed in metabolic standard cages placed in a controlled temperature room (20 °C) on a 12 h light–dark cycle and allowed free access to mice chow and tap water *ad libitum*.

2.4. Acute toxicity

The acute toxicity of strictosamide was evaluated i.p. according to the method of Reed and Muench as modified by Pizzi (1950). Male Charles River mice were divided in groups of six animals ($n = 6$). Strictosamide was dissolved in distilled water and graded doses were administered. After administration, the animals were observed for 48 h, and the specimens that survived or died were counted.

2.5. Hippocratic screening

Five groups of six intact and conscious male mice were administered i.p. with vehicle (water) or strictosamide in the doses 50, 100, 200 and 400 mg/kg body weight. The behaviour and physical appearance of the mice were observed prior i.p. injection at 5, 15, 30 min, 1, 3, 6 and 24 h after drug administration. The observations were conducted according to the guidelines of Malone and Robichaud (1962). The induced symptoms produced by each dose of strictosamide were noted on a 0–4 scale according to the intensity and duration of the effects.

Each hour, blood glycemia was determined by a kit of BM test glycemia and the rectal temperature was recorded using a digital thermometer. Urine samples of 6, 24 and 48 h were collected into cooled graduated cylinders and analysed by the COMBUR 10 test. All the effects of the drug were compared with the control group.

2.6. Na^+,K^+-ATPase preparation and assay

The animals were sacrificed by decapitation and exsanguinated. Brain and kidneys were immediately removed and placed in 20 volumes of ice-cold 0.32 M sucrose containing 1 mM EDTA. Each crude synaptosomal preparation was obtained by homogenation of a pool of 6 animal brains or kidneys in the sucrose solution. The homogenates were centrifuged at $1000 \times g$ at 4 °C for 10 min. The supernatants were removed and centrifuged at $12{,}000 \times g$ at 4 °C for 20 min (Gordon-Weeks, 1987; Maier and Costa, 1990). The pellets were resuspended in ice-cold 0.32 M sucrose containing 1 mM EDTA and used to determine ATPases activity according to Maier and Costa (1990). Total ATPase activity was determined spectrometricaly quantifying inorganic phosphorus produced by ATP hydrolysis and Mg^{2+}-ATPase activity was measured following a similar method in a K^+-free medium containing ouabain 1 mM. The difference of the two activities is taken as Na^+,K^+-ATPase activity. Inorganic phosphorus (Pi) was determined by a colorimetric assay of Taussky and Shorr (1953) and protein content was determined by the method of Lowry et al. (1951).

Inhibition curves of Na^+,K^+-ATPase activity using ouabain ranged from 0.1×10^{-3} to 0.1 mM were prepared and compared with the curves obtained using strictosamide ranged from 0.25 to 2 mg/mL, in order to determine the *in vitro* effects of this compound on Na^+,K^+-ATPase and Mg^{2+}-ATPase activities.

For the *in vivo* experiments, strictosamide was dissolved in distilled water (vehicle) and i.p. administered in single doses of 50, 100 or 200 mg/kg body weight to each group of six mice. The control group received i.p. vehicle. All the animals were sacrificed at 3 h of assay.

2.7. Statistical analysis

The 50% lethal dose (LD_{50}) was determined according the refered method (Pizzi, 1950). The results were expressed as means ± S.D. of six animals and the difference between groups were determined by ANOVA. Significance differences for $P < 0.05$ were analysed by the Duncan's test (Montgomery, 1991).

Table 1
Parenteral acute toxicity of strictosamide 48 h after i.p. administration.

Dose (mg/kg)	Dead ratio[a]	Mortality (%)
1000	18/18	100
900	12/14	86
800	8/13	62
700	5/14	36
600	3/16	19
500	1/19	5

[a] Accumulated number of dead mice/number of administered mice.

Table 2
Effect of strictosamide on rectal temperature and glycemia 6 and 3 h after i.p. administration, respectively.

Dose (mg/kg)	Rectal Δt °C after 6 h of assay	Δ[Glucose] mg/dL of blood after 3 h of assay
50	−1.16 ± 0.26[a]	16.5 ± 1.58[a]
100	−1.48 ± 0.12[a]	29.8 ± 3.08[b]
200	−3.16 ± 0.49[b]	45.5 ± 3.50[c]
400	−3.02 ± 0.32[b]	46.7 ± 3.17[c]

Values are expressed as mean ± S.D. from six animals. Significance was determined by ANOVA followed by Duncan's multiple range test. Groups bearing different superscript letters (a–c) are significantly different ($P < 0.05$).

3. Results

3.1. Pharmacological studies

Acute toxicity of strictosamide was evaluated after i.p. administration showing a LD_{50} = 723.17 mg/kg ($n = 6$), with the values 1.31 and 1.09 mg/kg representing the upper and lower confidence limits, respectively (Table 1).

As main effects produced by all doses of strictosamide in the pharmacological screening trials, we observed a slightly decrease of motor activity and muscular tone (2–3, in a 0–4 scale), depending on the dose. These effects were observed starting from 10, 7, 5 or 2 min and finishing by 25, 30, 38 or 50 min after the administration of 50, 100, 200 or 400 mg/kg of the strictosamide, respectively. With the higher doses of strictosamide, ataxia and hindlegs paralysis were also observed from 2 to 45 min after administration. Analysis of urine showed that pH and density had a maximum decrease, since 8–5.5 and since 1.1–1.0, respectively, during the first 6 h after administration of 400 mg/kg of strictosamide. Furthermore, during the same period, with this dose we observed the presence of glucose, blood, protein, ketones and bilirubin in urine. Strictosamide produced hypothermia and hyperglycemia with all doses, with the maxima results reported in Table 2.

3.2. Assays of Na^+,K^+-ATPase

As shown in Table 3, strictosamide significantly decreased the brain Mg^{2+}-ATPase activity at the 0.5, 1 and 2 mg/mL concentrations. Na^+,K^+-ATPase activity was nonsignificantly increased at the 0.25, 0.5 and 1 mg/mL concentrations but at the 2 mg/mL concentration it was also significantly decreased. Maximal significant decrease of Mg^{2+}-ATPase and Na^+,K^+-ATPase activities was 42.32% and 44.42%, respectively. Strictosamide, at all of the concentrations assayed, significantly decreased the kidney Mg^{2+}-ATPase activity, and produced a nonsignificant effect on Na^+,K^+-ATPase activity. Maximal percentage of Mg^{2+}-ATPase activity inhibition was 45.15%, observed at the 2 mg/mL concentration.

As shown in Table 3, i.p. administration of strictosamide at the doses of 50, 100 and 200 mg/kg significantly increased the brain Na^+,K^+-ATPase activity in a dose-independent manner and produced a slight nonsignificant decrease of Mg^{2+}-ATPase activity. Maximal percentage of Na^+,K^+-ATPase activity increase was 49.10% produced at the dose of 50 mg/kg.

Strictosamide, at the doses of 100 and 200 mg/kg, significantly decreased the kidney Mg^{2+}-ATPase activity (19.20%) but had nonsignificant effect on Na^+,K^+-ATPase activity compared with control.

4. Discussion

In the current work we studied the general activity of strictosamide on Charles River male mouse and the *in vitro* and *in vivo* effects of this compound on brain and kidney Na^+,K^+-ATPase and Mg^{2+}-ATPase activities.

The test for determination of acute toxicity indicated that strictosamide is moderately toxic to Charles River male mouse (LD_{50} = 723.17 mg/kg) when administered i.p. according to the classification of Hodgson (1997).

Results of hippocratic (observational) screening showed that all doses of strictosamide administered i.p. induced a decrease of muscular tone, indicating that strictosamide is a muscle relaxant. Furthermore, strictosamide decreased motor activity and rectal temperature, with the highest dose (400 mg/kg) producing ataxia and hindlegs paralysis, these effects showing strictosamide is a CNS depressor (Rothwell, 1992; Anthony et al., 2001).

The highest dose of strictosamide (400 mg/kg) produced the presence of glucose, blood, protein and bilirubin in urine. Furthermore, the highest doses of strictosamide (200 and 400 mg/kg) decreased urine pH from 8 (control) to 6 and 5.5, respectively. Kidney plays an important role in the excretion of metabolic wastes

Table 3
In vitro and *in vivo* effects of strictosamide on brain and kidney Na^+,K^+-ATPase and Mg^{2+}-ATPase activities of Charles River mice after 3 h of i.p. administration.

Groups	Na^+,K^+-ATPase (μmol *Pi* h^{-1} mg protein^{-1})		Mg^{2+}-ATPase (μmol *Pi* h^{-1} mg protein^{-1})	
	Brain	Kidney	Brain	Kidney
In vitro				
Control	4.66 ± 0.40[a]	2.50 ± 0.59	6.12 ± 0.28	8.46 ± 0.58
0.25 mg/mL	5.58 ± 0.95 (19.71%)	2.70 ± 0.65 (8.00%)	5.87 ± 0.32 (4.04%)	6.92 ± 0.35 (18.20%)*[a]
0.5 mg/mL	5.40 ± 0.58 (15.88%)	2.15 ± 0.71 (14.00%)	5.22 ± 0.36 (14.71%)*[a]	5.68 ± 0.14 (32.85%)*[b]
1 mg/mL	5.08 ± 0.97 (9.01%)	2.60 ± 0.83 (4.00%)	4.51 ± 0.54 (26.31%)*[b]	5.17 ± 0.15 (38.89%)*[b]
2 mg/mL	2.59 ± 0.04 (44.42%)*	1.58 ± 0.72 (36.80%)	3.53 ± 0.20 (42.32%)*[c]	4.64 ± 0.29 (45.15%)*[c]
In vivo				
Control	6.64 ± 0.87	5.52 ± 1.39	8.22 ± 0.29	11.46 ± 0.48
50 mg/kg	9.90 ± 0.72 (49.10%)*	6.94 ± 0.87 (25.72%)	8.21 ± 0.40 (0.12%)	11.74 ± 0.36 (2.44%)
100 mg/kg	9.11 ± 0.40 (37.20%)*	4.17 ± 1.44 (24.46%)	8.07 ± 0.26 (1.82%)	10.12 ± 0.53 (11.69%)*[a]
200 mg/kg	9.39 ± 0.64 (41.42%)*	4.78 ± 0.38 (13.41%)	7.87 ± 0.30 (4.26%)	9.26 ± 0.12 (19.20%)*[b]

Results are expressed as mean ± S.D. from six animals. Values in parenthesis indicate the percentage change vs. control. Significance was determined by ANOVA followed by Duncan's multiple range test. Within each group, means bearing different superscript letters (a–c) are significantly different at 0.05 level.

* $P < 0.05$ vs. control.

and in the regulation of extracellular fluid volume, electrolyte composition and acid balance. Many nephrotoxicants may reach toxic concentrations in the kidney and produce their primary effects on discrete segments or regions of the nephron, giving rise to functional abnormalities as impairment of renal concentrating ability or acidification defects. Our results, showing that i.p. administration of highest doses of strictosamide induced an increase of glucose, blood and protein urine levels and a decrease of urine pH, suggest that this compound produced nephrotoxic effects (Newman and Price, 2001; Schellmann, 2001). Since strictosamide is water soluble, these results show that this compound may account for kidney damage by *Sarcocephalus latifolius* infusions used in folk medicine.

Renal excretion of acid and conservation of bicarbonate occur through several mechanisms involving the extrusion of H^+ and accumulation of Na^+ by the Na^+-H^+ exchanger mechanism, and this process can be accompanied by the exchange of NH_4^+, K^+ and Na^+ driving by Na^+,K^+-ATPase (Heusel et al., 2001).

The Na^+,K^+-ATPase is a ubiquitous membrane-associated protein expressed in most eukariotic cells that maintains the high internal K^+ and low internal Na^+ concentrations typical of most animal cells. By coupling the energy released in the intracellular hydrolysis of one molecule of ATP, in the presence of Mg^{2+}, it extrudes three Na^+ from within the cell and accumulates two K^+ from outside, thereby maintaining concentration gradients of Na^+ and K^+ across the cell membrane. This electrochemical gradient provides energy for the transport of substrates and other ions across plasma membrane and it is essential also for regulation of cell volume and for the action potential of nerve and muscle. The Na^+,K^+-ATPase is expressed in a wide variety of tissues (e.g. skeletal and smooth muscle, heart, liver, kidney, intestine, exocrine glands and nervous tissue), in a tissue-dependent manner and it is subject to long- and short-term regulation by a variety of factors. Regulation of the Na^+,K^+-ATPase activity occurs by different cellular mechanisms and can be achieved by modulation of the number of enzyme molecules present at the plasma membrane or by influencing the activity of the Na^+,K^+-ATPase already at the cell surface. Modulation of Na^+,K^+-ATPase activity has been implicated in cell differentiation and growth and in several pathophysiologic processes, including cardiovascular, renal, neuronal, and metabolic disorders, having in common a dysfunction in salt and water homeostasis (Rose and Valdes, 1994; Dunbar and Caplan, 2001).

In the present study, we have investigated the *in vitro* and *in vivo* effects of strictosamide on brain and kidney Na^+,K^+-ATPase and Mg^{2+}-ATPase activity.

On the one hand, results of this studies showed that strictosamide had *in vitro* or *in vivo* nonsignificant effect on kidney Na^+,K^+-ATPase activity but induced a significant decrease of *in vitro* and *in vivo* Mg^{2+}-ATPase activity on this organ, in a concentration or a dose-dependent manner, respectively. Since Mg^{2+} is important for the modulation of Mg^{2+}-dependent enzymes, protein synthesis and cell growth, the decrease of Mg^{2+}-ATPase activity could produce low kidney intracellular Mg^{2+} ions concentrations and induce renal injury. Our results, showing that several *in vitro* concentrations and i.p. administration of different doses of strictosamide induced a decrease of kidney Mg^{2+}-ATPase activity, suggest the administration of this compound may produce nephrotoxic effects.

On the other hand, strictosamide significantly decreased the *in vitro* activity of brain Mg^{2+}-ATPase in the concentrations of 0.5, 1 and 2 mg/mL, but had no *in vivo* effect on Mg^{2+}-ATPase activity, these results suggesting that these effects are not relevant for the neurotoxicity produced after i.p. strictosamide administration. Furthermore, our results showed that the highest concentration of strictosamide (2 mg/mL) decreased the *in vitro* activity of Na^+,K^+-ATPase, but, on the contrary, i.p. administration of strictosamide produced a significant increase of brain Na^+,K^+-ATPase activity with all doses studied.

The Na^+,K^+-ATPase is composed of a catalytic α-subunit, (isoforms α_1, α_2, α_3 and α_4) and a β-subunit (β_1, β_2 and β_3). The α-subunit binds translocating cations and ATP. The β-subunit makes direct contact with the α-subunit, stabilising this subunit and assisting in its transport to the plasma membrane. In addition, β-subunit is important for ATP hydrolysis, ion transport, and the binding of inhibitor such as ouabain or other endogenous cardiac glycosides (Blanco and Mercer, 1998; Scheiner-Bobis, 2002). Regulation of Na^+,K^+-ATPase depends on many mechanisms, including regulation by substrates, membrane-associated components such as cytoskeletal elements and several circulating endogenous inhibitors as well as a variety of hormones, and moreover, regulation of Na^+,K^+-ATPase activity and its subcellular distribution depends on the effects of a range of specific intracellular signalling pathways, particularly involving protein kinases and phosphatases. The effect of modelators on Na^+,K^+-ATPase activity depends on isoenzymes composition and on the different isoforms affinity to the modelator (Therien and Blostein, 2000). It has been suggested that differences in cardiac glycoside sensitivity of Na^+,K^+-ATPase isozymes determine their therapeutic or toxic effects, such that the high affinity Na^+,K^+-ATPase mediates the positive inotropic effects of these glycosides whereas the low activity is responsible for its toxic effects (Maixent et al., 1987). Recent results demonstrated that the increase in heart mice contractility by low concentration of cardiac glycosides is mediated through the Na^+,K^+-ATPase α_2 isoform (Dostanic et al., 2003).

Results of present work showed that strictosamide, at certain concentration, it is an *in vitro* inhibitor, whereas *in vivo* it increases the activity of Na^+,K^+-ATPase, in a dose-independent manner. The activity of an enzyme may be measured by determining the rate of product formation or substrate utilization by mg of total protein used in the enzyme-catalysed reaction. Therefore, an increase or a decrease of enzymatic activity may be caused by a change of a specific property of the enzyme or by a change in the amount of enzyme within total protein (Zubay, 1993). In our study, we determined the Na^+,K^+-ATPase measuring the inorganic phosphorus produced by ATP hydrolyse during the enzymatic assays.

Results of *in vitro* assays reflect the direct physicochemical interactions between the mixed chemical and enzymatic system, including enzyme molecules, substrate and cofactors, whereas *in vivo* results leads not only with these but also with other possible interactions between the chemical and several cellular processes, including protein expression, targeting, membrane insertion and degradation. The amount of chemical that reaches the target cell depends on its physicochemical and pharmacokinetic proprieties affecting rate of penetration, transport mechanism from the administration site to the affected cells, and biotransformation in metabolites more active, less active, inactive or with different kind of activity (Lin and Lu, 1997; White, 2000).

The observation that chronic (Bluschke et al., 1976; Rayson, 1989; Kent et al., 2004) or nanomolar (Gao et al., 2002) administration of cardiac glycosides reversed the inhibition of Na^+,K^+-ATPase and increases its *in vivo* activity has already been reported by several authors, as well this effect it is associate with an increase in enzyme expression at certain experimental conditions.

The actual level of strictosamide in different brain cell type during our studies has not been assessed. Nevertheless, considering that chemicals which have some water solubility will distribute throughout body water whereas very lipophilic substances may became preferentially localized in fatty tissues, it is possible that only a very small amount of this compound reached the brain, in our study conditions, in consequence of the high solubility of

strictosamide in water. In fact, strictosamide could inhibit, either *in vitro* and *in vivo*, the Mg^{2+}ATPase in kidney, a organ highly perfused by blood, but it failed to inhibit *in vivo* Na^+,K^+-ATPase in brain, a lipid-rich organ, protected by a blood–brain barrier against the penetration of more polar or ionisable compounds. In consequence, a potential reason for the increase of *in vivo* brain Na^+K^+-ATPase activity produced after i.p. administration was that a small amount of strictosamide may stimulate rather than inhibit the Na^+K^+-ATPase activity in brain. However, considering that Na^+K^+-ATPase activity of treated groups was stimulated about 40% when compared with control group, it is possible that strictosamide may cause an isoform-specific induction of protein expression of brain Na^+K^+-ATPase and this effect may be the main reason of such activity increase.

On the one hand, results of current *in vitro* and *in vivo* studies showed that strictosamide have no effect on kidney Na^+,K^+-ATPase. These results suggested that strictosamide have no effect on the α_1 isoform of Na^+,K^+-ATPase, since this is the principal isoform of the kidney (Lucking et al., 1996). On the other hand, these studies demonstrated that strictosamide increased the brain Na^+,K^+-ATPase activity, this increase suggesting that strictosamide induced the α_2 isoform of Na^+,K^+-ATPase, since this is the most abundant isoform of the brain and the strictosamine did not produced effect on α_1 isoform (McGrail et al., 1991; Gick et al., 1993; Zahler et al., 1996; Peng et al., 1997).

The Na^+,K^+-ATPase from skeletal, cardiac and vascular smoth muscles, and that from brain are similar with respect that α_2 is the predominant isoform of these tissues. Thus, it is possible that any effect observed on brain Na^+,K^+-ATPase could also take place in some degree in refered muscular tissues. Acute inhibition of Na^+,K^+-ATPase activity or its synthesis decrease the net cellular uptake of K^+ and increase the retention of cellular Na^+.

Furthermore, this effect increases the muscle Ca^{2+} via the Na^+/Ca^{2+} exchanger and leads to the increase of muscle contractility. As a consequence of these effects, hyperkalaemic relaxion of skeletal muscle or increase in myocardial contractility or vasoconstriction could be produced. On the contrary, activation or increase in the concentration of Na^+,K^+-ATPase lead to the increase of cellular K^+, extrusion of cellular Na^+ and decrease of the muscle Ca^{2+} concentration, favouring the muscular contractile performance or the vasodilatation (Bova et al., 1990; Rose and Valdes, 1994; Clausen, 2003).

Blood pressure levels are controlled by complex combination of processes that influence cardiac output and peripheral vascular resistance (Rang et al., 2003). Since Na^+,K^+-ATPase contributes to myocardial contractility and vascular smooth muscle flexibility by modulation of Na^+ intracellular level, changes on Na^+,K^+-ATPase activity may be associate with the regulation of individual blood pressure level (Blaustein, 1993; Iwamoto and Kita, 2006). Taking in account that strictosamide is the main alkaloid from *Sarcocephalus latifolius* root, and reported results of our studies suggest strictosamide is inductor of α_2 isoform of Na^+,K^+-ATPase, we could speculate if strictosamide would not be responsible for the hipotensive action and the negative inotropic and chronotropic reversible effects produced in the frog and rabbit heart previously reported (Silva, 1964).

Several studies have suggested and supported the involvement of Na^+,K^+-ATPase on the pathogenesis of hypertension, showing a suppression of the cardiac α_2 isoform expression in different animal models of experimental hypertension, as compared to the normotensive controls. Moreover, the treatment with certain antihypertensive drugs that reduce the blood pressure to normal level reversed the referred suppression of α_2 isoform expression, which seems to be a pressure-sensitive isoform (Liu and Songu-Mize, 1997).

Results of our studies do not permit the elucidation of the precise molecular mechanism through which strictosamide increased the *in vivo* Na^+,K^+-ATPase activity. However, considering our results suggest the induction of α_2 isoform of cardiac Na^+,K^+-ATPase after strictosamide administration, in face of reported interactions between α_2 isoform and blood pressure level, we may consider that strictosamide favours the decrease of individual blood pressure level, independently this is a hypotensive effect of strictosamide produced by its action on molecular events cascade regulating α_2 isoform expression, or this is an antihypertensive effect of strictosamide inductive of pressure-sensitive factors that modulate such expression process. In consequence, and having also into account the high water solubility and high abundance of strictosamide in root, one may consider this compound may account to the traditional use of *Sarcocephalus latifolius* root aqueous extract as antihypertensive therapeutic agent.

In summary, the present results show that strictosamide is slightly toxic to Charles River male mouse, producing CNS depression and kidney toxicity, although the exact mechanism of these effects cannot be defined with certainty. Strictosamide inhibited the *in vitro* and the *in vivo* Mg^{2+}-ATPase activity on kidney but had no significant effect on brain. Furthermore, strictosamide had no effect on kidney Na^+,K^+-ATPase activity but produced an increase of *in vivo* Na^+,K^+-ATPase activity of brain, these findings suggesting that strictosamine may be inductor of α_2 isophorm of Na^+,K^+-ATPase and may account for the folk use of *Sarcocephalus latifolius* root infusion on hypertension. Further studies will be improved in order to study this hypothesis.

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