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# Trace elements cause oxidative damage in the brain of rats with induced hypotension

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ABSTRACT

Hypertension causes neuronal damage and apoptosis in the brain. Diazoxide is a drug used in the treatment of hypertension however, its effect on 5-hydroxyindole acetic acid (5-HIAA) and dopamine amines in adult animal models remains unclear. The purpose of this study was to determine the effect of oligoelements on 5-HIAA and dopamine in the brain of adult rats treated with diazoxide

*Methods:* Male Fisher rats (weight 250 g) were treated as follows: Group I, NaCl 0.9% (control); group II, tracefusin® (1.5 mL/rat); group III, diazoxide (20 mg/rat) and group IV, tracefusin® (1.5 mL/rat) + diazoxide (20 mg/rat). All doses were intraperitoneally administered on daily basis for four consecutive days. After the last administration, the brain of the animals was obtained and dissected in cortex, hemispheres (striatum) and cerebellum/medulla oblongata to measure the levels of 5-HIAA, dopamine, lipid peroxidation and total ATPase activity through validated methods.

*Results:* Dopamine and 5-HIAA levels decreased significantly in the group that received trace elements and diazoxide in the hemisphere regions, while in cerebellum/medulla oblongata, dopamine levels increased significantly in the groups that received diazoxide alone in. Lipid peroxidation in all brain regions increased significantly in the groups that received trace elements and diazoxide. ATPase dependent of calcium and magnesium decreased in the groups that received diazoxide alone or combined with trace elements in cerebellum/medulla oblongata regions.

*Conclusion:* The present results suggest that the use of trace elements and diazoxide alters metabolism of dopamine and 5-HIAA amines. Free radicals may be involved in this effect.

## 1. Introduction

Hypotension may increase the risk of Alzheimer-like pathological alteration and behavioral impairment through oxidative stress which leads to tau hyperphosphorylation and loss of dendritic spines (Liu et al., 2014).

In emergency situations, diazoxide is indicated for a short period in the management of blood pressure in hospitalized adults and children with severe and in adult malignant hypertension when urgent reduction of diastolic pressure is required (Zhang et al., 2014). However, its use for > 5 days is not recommended.

On the other hand, older adults have reduced vascular endothelial function, evidenced by attenuated nitric oxide (NO)-dependent cutaneous vasodilatation (Stanhewicz et al., 2015). Endogenous NO modulates the release of serotonin (Philippu, 2016), and is a neuromodulator as well, but an extra amount may lead to cell damage by oxidative stress or by forming nitroso-glutathione (NOGSH) within the cell (Hogg et al., 1996).

Since free radicals are known to damage cell components (Beckman et al., 1990), mainly plasma membrane lipids (Gutteridge and Halliwell, 1990), the central nervous system (CNS) is particularly susceptible, and extremely dependent on the amount of antioxidants, especially during development, when brain metabolism and growth rates are high (Driver et al., 2000). In patients attended in intensive care unit, it is common to see descriptions of some clinical conditions of oligoelement deficiency, even when the minimum daily requirements had been met. Henche Morilla et al. (1990), upheld that daily parenteral supplement of these elements should be higher than those recommended by the American Medical Association (AMA). Nevertheless Oligoelements like Zinc (Zn), Manganese (Mn), and Copper (Cu) which

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are essential for life, are integral parts of many important enzymes involved in a number of vital biological processes (Ozcelik and Uzun, 2009). Monoamine oxidase (MAO)-mediated neurodegeneration can result from the formation of hydrogen peroxide ($H_2O_2$) as a by-product of metabolism of aminergic neurotransmitters as biogenic amines in brain (Sies, 1993). Plasma membrane phospholipids in brain are in close contact with structural proteins that are embedded in the lipid bilayer (Swapna et al., 2005), from which, $Na^+$, $K^+$ ATPase is responsible of keeping the ionic interchange through this by the stimulation of $Na^+$ and $K^+$ flows (Stefanello et al., 2005).

There is a controversy concerning whether antioxidants might attenuate oxidative damage in humans after hypotension. In the face of this, it is necessary to determine the effects of oligoelements and diazoxide in order to establish methods for their safe administration. Thus, the aim of this study was to determine the effects of oligoelements and diazoxide on 5-HIAA, dopamine, lipid peroxidation and adenosine triphosphatase (ATPase) activity in the brain of adult rats.

## 2. Material and methods

Male Fisher rats (weight 250 g) were treated as follows: Group I, NaCl 0.9% (as control); group II, tracefusin® (1.5 mL/rat); group III, diazoxide (20 mg/rat) and group IV tracefusin® (1.5 mL/rat) + diazoxide (20 mg/rat). N = 6 rats per group. All doses were intraperitoneally administered on daily basis for four consecutive days.

Subsequent to the administration of the last dose, the brain of the animals was extracted and sagittally dissected. The left cut was homogenized in 5 volumes of TRIS-HCl 0.05 M [pH 7.4] and used to measure lipid peroxidation and ATPase activity. The right cut was homogenized in 5 volumes of perchloric acid ($HClO_4$) 0.1 M and employed in the evaluation of 5-HIAA and dopamine levels.

It is worth mentioning that each 100 mL of oligoelemental solution (tracefusin®) contains zinc (55 mg), copper (16.90 mg), manganese (38.10 mg), sodium (163.9 mg), fluoride (14 mg), iodide (1.3 mg) and chloride (25.6 mg).

### 2.1. Levels of 5-HIAA

The levels of 5-HIAA was measured from the perchloric acid homogenate based on the method proposed by Beck et al. (1997). 1.9 mL of 0.01 M acetate buffer with a pH 5.5 and containing a tissue homogenate aliquot was incubated at room temperature for 5 min under a total darkness. Samples were analysed in a Perkin Elmer LS 55 spectrofluorometer with excitation/emission wavelength of 296 nm/333 nm, using an FL Win Lab software version 4.00.02. A previously constructed standard curve was used to read the results.

### 2.2. Measurement of dopamine (DA)

The levels of DA were measured from the supernatant of tissue homogenized in $HClO_4$ after centrifugation at 9000 rpm for 10 min in a microcentrifuge (Hettich Zentrifugen, model Mikro 12-42, Germany), with a version of the technique reported by Calderón et al. (2008). An aliquot of the $HClO_4$ supernatant, and 1.9 mL of buffer (0.003 M octylsulphate, 0.035 M $KH_2PO_4$, 0.03 M citric acid, 0.001 M ascorbic acid), were placed in a test tube. The mixture was incubated for 5 min at room temperature in total darkness, and subsequently, the samples were read in a spectrofluorometer (Perkin Elmer LS 55, England) with 282 nm excitation and 315 nm emission lengths. FL Win Lab version 4.00.02 software was used. Values were calculated from a previously standardized curve and reported as μM/g of wet tissue.

### 2.3. Measurement of lipid peroxidation (Tbars)

Determination of thiobarbituric acid reactive substances (Tbars) as indicator of lipid peroxidation was carried out according to the method of Gutteridge and Halliwell (1990). Each tissue sample was homogenized in 3 mL of phosphate buffer pH 7.4, from which 0.5 mL aliquot was taken and added to 1.5 mL of thiobarbituric acid (TBA) solution containing TBA (1.25 g), trichloroacetic acid (40 g) and concentrated HCl (6.25 mL) dissolved in 250 mL deionised water. The whole mixture was heated to water boiling point for 30 min (Thermomix 1420). Samples were then put in an ice bath for 5 min and then centrifuged at 3000 g for 15 min (Sorvall RC-5B Dupont). Supernatant absorbance was spectrophotometrically read in a three-set scheme at 532 nm (Heλios-α, UNICAM). The concentration of MDA was calculated by the absorbance coefficient of MDA–TBA complex ($1.56 \times 10^5\ M^{-1}\ cm^{-1}$). The Tbars concentration was expressed as malondialdehyde μmoles per gram of wet tissue.

### 2.4. Measurement of total ATPase

The activity of ATPase was assayed according to the method proposed by Calderon et al. (2013). 1 mg (10%) w/v of homogenized brain and heart tissues in tris-HCl 0.05 M pH 7.4 was incubated for 15 min in a solution containing 3 mM $MgCl_2$, 7 mM KCl, and 100 mM NaCl. To this was added 4 mM tris-ATP and incubated for another 30 min at 37 °C in a shaking water bath (Dubnoff Labconco). 100 μL 10% trichloroacetic acid w/v was used to stop the reaction and samples were centrifuged at 100 g for 5 min at 4 °C. Inorganic phosphate (Pi) was measured in triplicates using one supernatant aliquot as proposed by Fiske and Subbarow (1925). Supernatant absorbance was read at 660 nm in a Helios-α, UNICAM spectrophotometer and this absorbance was then expressed as mM Pi/g wet tissue per minute.

### 2.5. Analysis of results

Kruskal-Wallis and ANOVA tests were used with their corresponding contrasts, and previous variance homogeneity comparison. Values restricted to $p < 0.05$, were considered statistically significant (Castilla-Serna, 2011). JMP Statistical Discovery from SAS version 8.0.0 software was used.

## 3. Results

The blood pressure and cardiac frequency were registered in all groups studied as indicators of their treatment. Data analysis revealed no effect attributable to any of the treatments administered over the blood pressure or cardiac indicators (Table 1).

### 3.1. Dopamine

Dopamine concentration decreased in hemispheres (striatum) in the group of animals treated with trace elements + diazoxide as compared with saline, trace elements and diazoxide treated groups; however, this decrement was statistical different ($p < 0.0003$) only versus saline, and diazoxide groups. In contrast an increment was observed in cerebellum/medulla oblongata ($p < 0.008$) for those animals where diazoxide was administered in comparison with saline solution or trace elements + diazoxide groups. No differences were observed in cortex

**Table 1**
The blood pressure and cardiac frequency registered in rats treated either with trace elements, diazoxide or both.

| Treatment | Systole | Diastole | Cardiac frequency |
|---|---|---|---|
| Saline | 105.3 ± 5 | 77.7 ± 9 | 75.0 ± 10 |
| Trace elements | 107.6 ± 7 | 78.4 ± 8 | 71.3 ± 8 |
| Diazoxide | 103.9 ± 9 | 69.7 ± 10 | 75.4 ± 10 |
| Trace elements + diazoxide | 104.6 ± 9 | 76.6 ± 8 | 72.1 ± 7 |

Blood pressure (mm Hg), cardiac frequency (bpm).