REGIONAL OXIDATIVE STRESS IN ENCEPHALON OF FEMALE MICE WITH POLYPHENOLIC EXPOSURE FROM TEA EXTRACTS IN ORAL OVERWEIGHT PLANT-BASED TREATMENT

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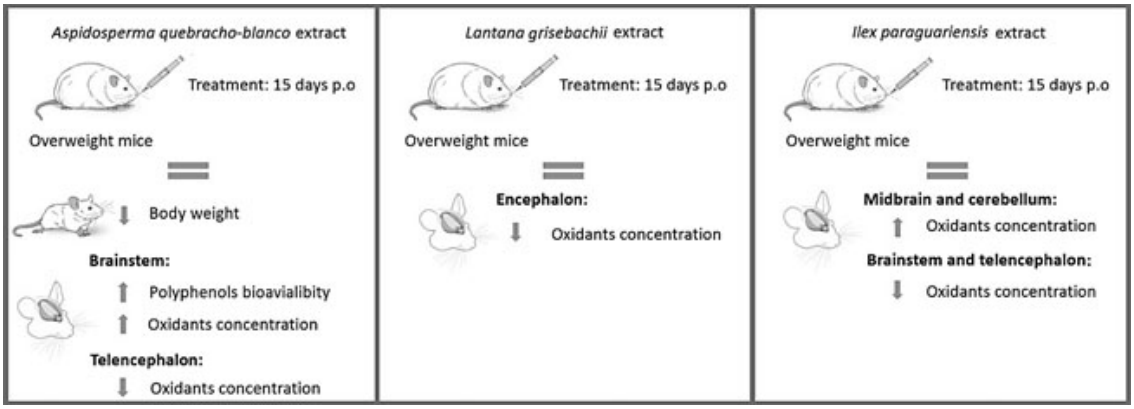
Abstract

Polyphenols provide by diet may act as antioxidant in the Central Nervous System and exert a protective effect on metabolic diseases. The aim of this study was to establish tea extract effects on oxidative status and murine overweight in accordance with polyphenolic availability in different encephalic regions. Methods: Balb/c mice (female, n>3) with overweight received for 15 days 100 mg/Kg/d of extract from Lantana grisebachii, Aspidosperma quebracho-blanco, or Ilex paraguariensis extracts and control group (received water without extract). Body weight gain was recorded regularly. Polyphenols, hydroperoxides (HP), lipid peroxides (LP), and superoxide anion (SO) were measured in brain (telencephalon and diencephalon), midbrain, brainstem and cerebellum. Results were compared by ANOVA followed by the Tukey test (P<0.05). Results: A. quebracho-blanco-based treatment decreased weight gain and increased polyphenols in brainstem (p<0.02), although it concomitantly increased SO and LP in this region (p=0.0029 and p=0.0280, respectively). L. grisebachii-based treatment reduced oxidative markers differentially in each region (p<0.05). I. paraguariensis-based treatment oxidized midbrain and cerebellum, although it was antioxidant in the brainstem (p<0.05). All treatments were antioxidant in telencephalon (p=0.0029). Conclusions: The A. quebracho-blanco extract was active on overweight and increased polyphenols in brainstem, with safe functional derivatives being required to avoid oxidative stress. Other extracts affected oxidative status in a region-dependent manner.

Graphical abstract



1. Introduction

Plant polyphenols are xenohormetic compounds with health potential [1], which are provided by vegetable foods (e.g. fruits, vegetables, cereals, olives, vegetables, cocoa, coffee etc.) and drinks, such as teas or infusions [2]. Then, they cross blood-brain barrier and enter in the central nervous system [3], to exert several redox and different regulative effects [4]. Given this, they prevent the development of chronic non-communicable diseases, such as overweight and obesity, involving redox-related chronicity [5]. Thus, bioprospecting in American flora can provide some bioactive antioxidant polyphenols to be included as nutritional or pharmacological agents for the control of these health problems, which elevated morbidity and mortality worldwide [6].

Organic regulation of body weight depends on the participation of different brain regions (cerebral cortex, hypothalamus and brainstem), and other interrelated systems and their mediators (e.g. digestive and endocrine ones)[7]. In this sense, polyphenols extracted by plant infusion are available natural compounds, which can modulate functions of these systems[8]. Furthermore, their tissue concentrations in telencephalon and diencephalon are inversely correlated to body weight gain (unpublished results). Nonetheless, polyphenols need to be analyzed by a pharmaco-nutritional approach to assess their sources, bioavailability, desirable and undesirable effects [3,9], in order to discern between their positive and toxic activities [10].

Previous reports support selection of the following plants to assay their extracts [11,12]: Lantana grisebachii Stuck. (Verbenaceae) (LG), Aspidosperma quebracho-blanco Schltdl. (Apocynaceae) (AQB), and Ilex paraguariensis St.-Hil. (Aquifoliaceae) (IP). Thus, polyphenol bioavailability and oxidative effects of them were measured in different encephalic regions to be related to weight control.

2. Material and Methods

2.1. Equipment and materials

Fast Blue BB salt (4-benzoylamino-2,5-dimethoxybenzenediazonium chloride hemi-(zinc chloride)), xylenol orange, and other chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Solvents were obtained from Cicarelli SA (Argentina). Spectrophotometric readings were performed with a GloMax® Multi Microplate Multimode Reader (Promega Corp., Madison, WI, USA).

2.2. Plant extracts

Wild LG and AQB were collected during summer at -31.28/-64.44 GPS coordinates (mountain zone of the phytogeographic Chaquenian region, Cordoba, Argentina), with identified specimens being in the RIOC Herbarium of Argentina. Commercial IP was obtained from organic cultures. One gram of pulverized air-dried samples was extracted by adding ten mL of water heated at 95°C. These infusions were allowed to cool at room temperature for 1 hour in darkness under constant shaking. Then, they were centrifuged to recover extracts from each supernatant by filtration and 24-h freeze-drying. Phytochemical contents were previously reported [11].

2.3. Experimental conditions

This study was carried out according to the ethical and technical US guidelines, with the approvement of the Institutional Committee for the Care and Use of Laboratory Animals (National University of Cordoba, Argentina). Animals were bred under standard laboratory conditions and fed ad libitum with commercial diet (200±13 g/kg/d; Cargill SACI, Argentina) and potable water (150±10 mL/kg/d; Aguas Cordobesas SA, Argentina). One-month-old Balb/c female mice with a 20% of body weight over the mean expected for age 13, were separated into four experimental groups to be treated orally for 15 days: C (water without extract), AQB, LG, and IP (100 mg/kg/day of each water-dissolved extract, equivalent to the consumption of ~2 L of infusion in humans). General status, body weight, food, and water consumption were controlled. Then, they were sacrificed. Organs were weighed and divided into different regions: brain (telencephalon and diencephalon), midbrain, brainstem and cerebellum. These tissues were mechanically homogenized in 1.25 mL of 60% methanol with 62.5 µL of 50% trichloroacetic acid. After 30-min incubation at at 50°C in darkness to obtain polyphenols and oxidants 12, supernatants were recovered by 5-min 10,000-rpm centrifugation to perform assays with them.

2.4. Polyphenols

Acid-methanolic supernatants of regions were mixed with a 0,1% Fast Blue BB and 20% sodium bicarbonate (volume relation 10:1:1), for 30 min in

darkness at 37ºC. Then, polyphenols were measured at 450 nm, to calculate gallic acid equivalent µg per gram of tissue using a standard curve (0.01-18.75 µg of gallic acid; Anedra, Argentina). Results were expressed as percentages respect to control (%).

2.5. Oxidants

They were measured as previously done in central nervous system [12], with absorbances being standardized per tissue gram and calculated as percentages respect to control (%). First, superoxide anion (SO) of samples reacted 30 min in darkness at 37ºC with 0.1% nitroblue tetrazolium (volume relation 9:1). Then, 2 M potassium hydroxide and dimethylsulfoxide were added to the reaction (volume relation 1:1:2). Absorbance was recorded at 600 nm. On the other hand, hydroperoxides and lipid peroxides (HP and LP, respectively) of samples reacted 30 min with solution A (HP: 100 mM sorbitol and 125 µM xylenol orange; or LP: 4 mM butylated hydroxytoluene and 125 µM xylenol orange in 90% methanol) and B (25 mM ferrous ammonium sulfate in 2.5 M sulfuric acid) (volume relation 10:100:1). Absorbance was recorded at 540 nm.

2.6. Statistical analysis

Data were expressed as mean ± standard error (SE). ANOVA models followed by the Tukey test were used to compare treatment effects (C, AQB, LG, and IP) on body weight and encephalic variables (tissue concentrations of polyphenols and oxidants), with $P<0.05$. Spearman coefficients (SC) were utilized to evaluate correlation. The InfoStat v.2012 software was used (InfoStat Group, Argentina).

3. Results

Mice showed the following body weight gain after 15 days of treatment: C: 2.80±0.20 g, AQB: -0.23±0.62 g, IP: 1.60±0.10 g, and LG: 1.47±0.20 g. This meant a weight gain reduced by the three treatments, with AQB showing a significant effect ($p<0.05$) (Figure 1). Concerning this, AQB increased polyphenols in telencephalon, diencephalon, and significantly brainstem ($p=0.0062$). Other experimental groups showed control levels of these compounds in the different studied encephalic regions (Figure 2).

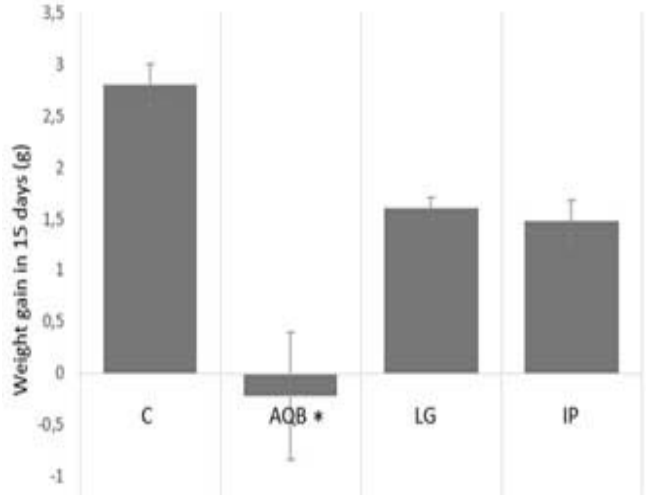


Figure 1. Weight change of Balb-c mice treated during 15 days with 100 mg/kg/day (mean ± SE of n≥3 expressed as percentages respect to C). Experimental groups compared by ANOVA (*p<0.05): C: Control (untreated); AQB: A. quebracho-blanco; IP: I. paraguariensis; LG: L. grisebachii.

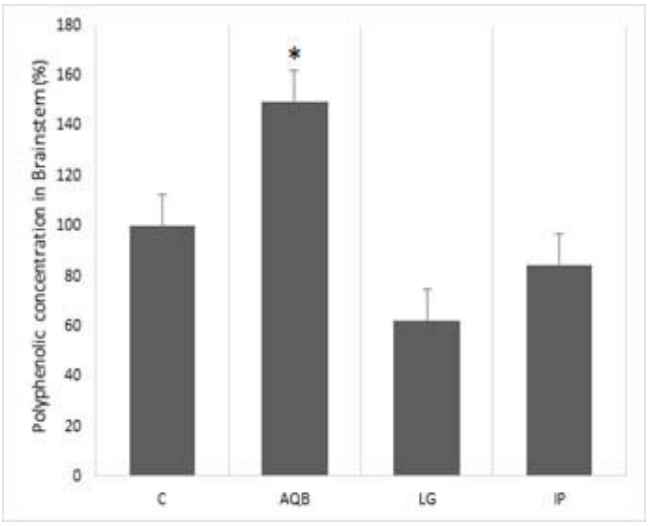


Figure 2. Polyphenols in brainstem of Balb-c mice treated during 15 days with 100 mg/kg/day (mean ± SE of n≥3 expressed as percentages respect to C). Experimental groups compared by ANOVA (*p<0.05): C: Control (untreated); AQB: A. quebracho-blanco; IP: I. paraguariensis; LG: L. grisebachii.

In telencephalon, LG, IP and AQB decreased lipid peroxides respect to C ($p=0.0029$) (Figure 3a). In midbrain, IP increased lipid peroxides and superoxide anion respect to C ($p=0.0009$ and $p=0.0001$, respectively), whereas LG reduced superoxide anion ($p=0.0001$). AQB showed no significant effect (Figure 3b). In brainstem, AQB increased superoxide anion and lipid peroxides ($p=0.0029$ and $p=0.0280$, respectively), whereas LG and IP reduced hydroperoxides ($p=0.0022$)

(Figure 3c). In cerebellum, IP induced lipid peroxides (p=0.0008), whereas LG decreased hydroperoxides (p=0.0066) (Figure 3d). No additional changes in oxidant levels were found in encephalon.

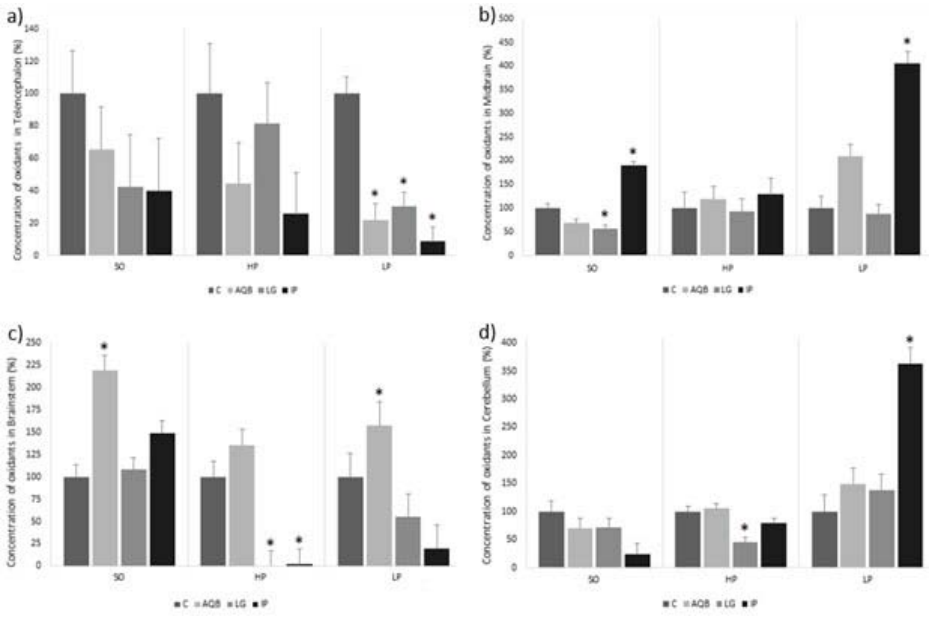


Figure 3. Oxidants in central nervous system of Balb-c mice treated during 15 days with 100 mg/kg/day (mean ± SE of n≥3 expressed as percentages respect to C). Experimental groups compared by ANOVA (*p<0.05): C: Control (untreated); AQB: A. quebracho-blanco; IP: I. paraguariensis; LG: L. grisebachii. Abbreviations: SO: Superoxide anion; HP: hydroperoxides; LP: lipid peroxides.

4. Discussion

Although infusions from A. quebracho-blanco, I. paraguariensis and L. grisebachii are able to modulate encephalic redox status of healthy mice [12], their relevance in pathological conditions was unknown. Thus, they were assayed in mice with overweight, with own data suggesting their content of bioactive compounds.

Only the AQB extract acted as a significant source of phenolic compounds able to accumulate in central nervous system, by increasing these neurotropic polyphenols in the brainstem and midbrain in a lesser extent, which was associated with reduced weight gain. Accordingly, plant polyphenols modulate physiology to counteract obesity [14], involving hypothalamus, hippocampus and brainstem [15,16]. Furthermore, this region showed the highest polyphenolic concentration, which might include flavonoid aglycones [17]. Thus, they are candidates as neural and metabolic regulators [18,19].

AQB treatment showed an antioxidant effect on telencephalic region associated with the reduction in weight gain. In this regard, experimental evidence establish that adequate overweight control, brain redox state and health benefits are related [20,21]. However, this should be criticized, as the antioxidant activity could be temporary and/or not having clinically relevant effects. In fact, antioxidant response to the LG extract is toxic after long-term use [12]. Similarly, treatment with IP showed regional differences (antioxidant in telencephalon and brainstem, but oxidant in cerebellum and midbrain). Moreover its antioxidant effect is transitory [12]. Therefore, this context, redox changes induced by phytochemicals are not sufficient to guarantee neuroprotection and neuroregulation.

Therapeutic AQB potential is limited by the induction of oxidative stress in the brainstem, a side effect, which can be enhanced by prolonged use [12]. Moreover, this oxidative effect was found outside nervous system (unpublished results). Hence, it is necessary to identify and differentiate compounds responsible for its desirable and undesirable effects. This toxicity has been recognized [22]. However, data is contradictory [23,24], which have been also seen by nutritional epidemiology [25].

5. Conclusions

In conclusion, AQB reduced weight gain in female mice in a better way than LG and IP, which was related to regional redox changes in encephalon. Nonetheless, oxidative stress was a side effect, which must be considered to obtain their phytochemicals to treat overweight and obesity, a major concern for human health.

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Conflict of interest
None of the authors have any conflicts of interest related to this manuscript.

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