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Data Article

Data of the effects of acetone fraction from *Sechium edule* (Jacq.) S.w. edible roots in the kidney of endothelial dysfunction induced mice

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ABSTRACT

Endothelial dysfunction induced by Angiotensin II (AG II) plays an important role in the pathogenesis of hypertension and is accompanied by a prooxidative condition, which in turn induces an inflammatory state, vascular remodeling, and tissue damage including the kidney (Schmitt and Dirsch, 2009) [1]. New drugs that can control several of these pathologies are required. *Sechium edule* has been reported to possess antioxidant, anti-inflammatory and antihypertensive activity (Ibarra-Alvarado et al., 2010) [2]. This

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paper contains data complementary to those published in Journal of Ethnopharmacology (Moreno et al., 2018) [3], evaluating the effect in kidney of hypertensive mice of the acetone fraction from *S. edule* to control de pro-oxidative state, reduction of the inflammatory adhesion molecule (ICAM) and recruitment of inflammatory cells.

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Specifications Table

Subject area More specific subject area Type of data How data was acquired	Medicine Use of extracts of <i>Sechium edule</i> to control endothelial dysfunction Text file and figures The Activity of antioxidant enzymes was measured by enzymatic reactions measuring the end product using a Spectrophotometer and analyzed in Excel and Microphotographs were acquired with a e Nikon ECLIPSE 80i microscope. The images were analyzed with the Metamorph software, v. 6.1.survey.
Data format	Analyzed
Experimental factors	The acetonic fraction of <i>Sechium edule</i> roots (rSe-ACE) were obtained and tested in its anti-oxidant, anti-inflammatory and anti-hyperten- sive capacity in a murine model of endothelial dysfunction [3].
Experimental features	Hypertension was induced in female mice by the chronic adminis- tration of angiotensin II for 10 weeks [1]. Treatments (Losartan or the acetonic fraction of <i>Sechium edule</i>) were coadministrated also for the 10 weeks. At the end of the experiment, kidneys were obtained and processed for biochemical or microscopic studies
Data source location	<i>Sechium edule</i> (Jacq.) Sw. roots were collected in the community of Tuxpanguillo, Veracruz, Mexico (18°47′00.5″N, 97°00′17.5″W, at 1721 m above mean sea level) in a period from April through May.
Data accessibility	Data is with this article.

Value of the data

- A model of endothelial dysfunction is induced in rats by the chronic administration of angiotensin II.
- rSe-ACE is able to control Endothelial dysfunction (ED) and its associated pathologies.
- rSe-ACE is a good candidate for the development of a phytochemical medicine for ED.

1. Data

The dataset of this article provides information on the effects of the acetonic fraction of *Sechium edule* roots in the activity of antioxidant enzymes (Fig. 1), in the expression of the inflammatory adhesion molecule ICAM 1 (Fig. 2) and in the recruitment of inflammatory cells on renal capsule (Fig. 3) in kidney of endothelial dysfunction induced mice through the administration of Angiotensin [1].



Fig. 1. Effect of rSe ACE on renal activity of antioxidant enzymes in mice. Data are expressed as mean \pm SD and were analyzed by ANOVA and a post-hoc Tukey-Kramer test. *Statistically significant differences with respect to control (P < 0.05).

2. Material and methods

2.1. Animals and experimental groups

Three groups of hypertensive female C57BL/6J mice, five mouse each, were coadministered with losartan (10 mg/kg) diluted in carboxymethylcellulose (CMC) 0.2% [4] or rSe-ACE (10 mg/kg) [2] diluted in water orally for 10 weeks. A fourth group was treated only with saline solution. Mice were regarded as hypertensive when blood pressure increased by 15% or more with



Fig. 2. ICAM-1 expression by immunohistochemistry. ICAM-1 expression in Renal capsule, Cortical glomerulus and Perirenal fat tissue of control intact mice and of AgII treated mice with or without Losartan (LOS) or rSeACE. 40× microphotographs. m: mononuclear cells.



Fig. 3. Cell populations in the renal capsule. Cellular infiltrate in the renal capsule from the control mice and from the AGIItreated group further treated with or without Losartan or rSeACE. B: basophile; F: fibroblast; L: lymphocyte; N: neutrophil; Macrophage; P: plasma cell. Micrographs were taken at 100X.

respect to baseline blood pressure (systolic or diastolic). Mice were killed by CO₂ inhalation and exsanguinated at the end of the experiment

2.2. Tissue preparation for biochemical analysis

Kidneys were homogenized in ice-cold HEPES buffer (HEPES 25 mM, EDTA 1 mM, and phenylmethylsulfonyl fluoride [PMSF] 0.1 mM). Homogenates were centrifuged at $6000 \times g$ for 5 min at 4 °C and supernatants were recovered. Protein concentration was determined by the Lowry method.

2.2.1. Measurement of O_2^- production

Dihydroethidium oxidation (DHE) to ethidium (Eth) was used to measure O_2^- production. Supernatants (10 µl) were incubated with DHE (0.02 mM), salmon testis DNA (0.5 mg/mL), and the corresponding substrate for xanthine oxidase (XO), mitochondrial respiratory enzymes, or nitric oxide synthase (NOS). Eth-DNA fluorescence was measured at an excitation wavelength of 480 nm and an emission wavelength of 610 nm at 37 °C for 30 min using a multimode microplate reader

(Synergy HT, Biotek, Winooski, VT). Xanthine (0.1 mM) was used as a substrate for XO, and allopurinol (0.2 mM) was used as an inhibitor. Succinate (5 mM) was used as a substrate for mitochondrial O_2^- production, and antimycin (0.05 mM) was used to block the respiratory chain. L-arginine (L-Arg, 1 mM) was used as a substrate for NOS, while NG-nitro-L-arginine methyl ester (L-NAME, 1 mM) was used to block NOS activity. A blank without sample was run to subtract background fluorescence from each sample reading. Enzyme activity is expressed with respect to control readings.

2.2.2. Glutathione peroxidase assay

The activity of renal glutathione peroxidase (GPx) was assayed by measuring NADPH disappearance by optical density (OD) reading at 340 nm. GPx uses GSH to reduce tert-butyl hydroperoxide, thereby producing GSSG, which is readily reduced to GSH by GR using NADPH as a reducing equivalent donor. Blank reactions with the sample replaced by distilled water were sub-tracted from each assay reading. Data were expressed as units per mg protein.

2.2.3. Glutathione reductase assay

The activity of glutathione reductase (GR) was assayed using oxidized glutathione as a substrate. Briefly, GR reduces GSSG to GSH at the expense of NADPH; the disappearance of NADPH can be detected by reading OD at 340 nm.

2.2.4. Glutathione-S-transferase assay

The activity of glutathione-S-transferase (GST) was evaluated using a reaction mixture consisting of potassium phosphate buffer 50 mM (pH 6.5), reduced glutathione 2 mM, and 1-chloro-2,4-dinitrobenzene (CDNB) 1 mM. Supernatants (0.02 ml) were added to 0.98 ml of the reaction mixture, and absorbance was recorded at 340 nm for 3 min. Enzymatic activity was calculated as μ mol CDNB conjugate formed/min/mg protein using a molar absorptivity of 9.6 × 10³/M/cm.

2.2.5. Superoxide dismutase assay

The total activity of Superoxide Dismutase (SOD) was evaluated employing a competitive inhibition assay using a xanthine-XO system to reduce nitroblue tetrazolium (NBT). The percentage of NBT reduction in the sample-free control tube was 100%. The amount of protein that inhibited NBT reduction by 50% of the maximum reading was defined as one unit of SOD activity. Results were expressed as U/mg protein.

3. Histopathology and immunohistochemistry

After the last blood pressure measurement, mice were anesthetized with sodium pentobarbital (30 mg/kg i.p.) and perfused with ice-cold PBS (NaCl 140 mM, KCl 2 mM, and K₂HPO₄ 1.15 mM). Kidneys were removed. The organs were fixed in Zamboni solution (formaldehyde 2.0%, picric acid 0.2%, pH 7.0). Tissues were then dehydrated and embedded in paraffin. Tissue sections (5 μ m) were transferred to poly-L-lysine-coated slides (Sigma) before being deparaffinized and rehydrated. For histopathological studies, the slides were stained with PAS stain. For Immunohistochemistry, kidney slides were deparaffined, rehydrated, and incubated with H₂O₂ 3%, albumin 5%, and Tween 20-PBS 1%. After treatment, tissue sections were incubated overnight with rat anti-mouse ICAM-1 (eBioscence) diluted 1:100 in albumin 0.1% and Tween 20-PBS 0.05%. After wash with PBS, tissues were incubated with 50 μ l of biotinylated goat anti-rat IgG (MP Biomedicals) antibody, followed by HRP-labeled streptavidin solution (MP Biomedicals) at 37 °C for 30 min, and developed with 3,3 diaminobenzidine (ZYMED, San Francisco, CA). The slides were counterstained with hematoxylin and photographed using a Nikon ECLIPSE 80i microscope. The images were analyzed with the Metamorph software, v. 6.1.

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Transparency document. Supplementary material

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