Regular Article

Effect of *Ocimum basilicum*, *Ocimum selloi*, and Rosmarinic Acid on Cerebral Vascular Damage in a Chronic Hypertension Model

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The main objective of treatment against hypertension is not only to reduce blood pressure levels, but also to reduce vascular risk in general. In the present work, administering angiotensin II (AGII; 0.2 μg/kg intraperitoneally (i.p.) for 12 weeks) activates the hypothalamic–pituitary–adrenal (HPA) axis, which caused an increase in corticosterone levels, as well as in proinflammatory cytokines (interleukin 1β (IL-1β), interleukin 6 (IL-6), and tumor necrosis factor alpha (TNF-α)) and macrophage chemotactic protein 1 (MCP-1), and decreased anti-inflammatory cytokines (interleukin 10 (IL-10) and interleukin 4 (IL-4)). On observing the behavior in the different models, an anxiogenic effect (elevated plus maze (EPM)) and cognitive impairment (water Morris maze (WMM)) was observed in animals with AGII. By administering organic extracts from *Ocimum basilicum* (Oba-EtOAc) and *Ocimum selloi* (Ose-EtOAc), and some doses of rosmarinic acid (RA) (6 weeks *per os* (*p.o.*)), the damage caused by AGII was stopped by re-establishing corticosterone serum levels and by decreasing the proinflammatory cytokines and MCP-1.

Key words *Ocimum basilicum*; *Ocimum selloi*; angiotensin II; inflammation; anxiety; memory

INTRODUCTION

Chronic arterial hypertension (AH) alters the structure of the cerebral vasculature; thus, the vasoregulatory mechanisms controlled by a functional endothelium are also disrupted. Blood supply to the brain decreases and also increases the susceptibility of this organ to ischemic injury, which causes neuronal death and loss of synapses in regions involved in learning and memory processes.[1,2] An important element in the pathophysiology of cardiovascular and cerebrovascular diseases is the over-activation of the renin angiotensin aldosterone system (RAAS).[3,4] One of the main consequences of this altered mechanism is the sustained production of angiotensin II (AGII), one of the final products and the main mediator of this system. The increase in AGII first generates AH, but also induces damage and vascular remodeling, including sustained vasoconstriction, oxidative stress, the inflammatory process, the pro-coagulant state, and cell proliferation.[3,5–7]

On the other hand, over-activation of RAAS gives rise to a state of anxiety associated with AH. The anatomical substrate that explains this linkage is the hypothalamic–pituitary–adrenal (HPA) axis, which is the body's main response system to emotional stress.[8–11] It has been shown that increased flow through the small arteries, derived from AH, promotes medial hypertrophy (of endothelial and vascular smooth muscle cells),[5] due to the vascular inflammation that underlies the development of AH.[12,13] In parallel, there is an increase in the production of superoxide radical; consequently, there is an infiltration of the leukocytes and other cellular responses that involved an increase of inflammatory cytokines, chemokines, growth factors, and matrix metalloproteinases.[3,6,13–15] This generates a vicious circle of anxiety–hypertension–inflammation–oxidative stress–vascular damage–neurological damage.

In this chronic-degenerative process, oxidative stress through the reactive oxygen species (ROS) acts as a signal that induces the expression of messengers for the synthesis of transcriptional factors, which include nuclear factor kappa B (NF-κB) and the activating protein AP-1. Both of the latter are directly involved in the pathogenesis of vascular damage and, in turn, the direct synthesis and release of tumor necrosis factor alpha (TNF-α), and certain interleukins and chemokines such as macrophage chemotactic protein 1 (MCP-1).[6,13]

Therefore, vascular diseases are considered an emerging risk factor for dementia and cognitive decline.[16] Because of all of the above, the brain becomes a principal target of the harmful effects of AH and is a cause of related mortality and morbidity.[1]

Species of the genus *Ocimum* have an extensive ethnomedical and culinary use. They contain secondary metabolites useful in the treatment of chronic degenerative diseases.[17] *O. basilicum*, for example, has been widely used by the pharmaceutical and cosmetic industry due to its high content of rosmarinic acid (RA), an ester of caffeic acid, with antioxidant, anti-inflammatory, antibacterial, and antiviral ac-

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tivities.[18] Another example of this genus is *O. selloi*, which is native to Mexico, whose pharmacological effects have not, to our knowledge, been studied, although the presence of the following essential oils has been described for this plant: methyl chavicol or stragole; *trans*-anethole; *cis*-anethole, caryphyllene,[19] and linalool.[17]

The objective of the present work was to evaluate the effect of standardized ethyl acetate extract in its RA content, one of *O. basilicum* (Oba-EtOAc), and the other of *O. selloi* (Ose-EtOAc), in addition to evaluating the effect of different doses of RA on the following: the behavior of anxiety and memory; the serum concentration of corticosterone; the renal and cerebral levels of MCP-1, and interleukin 1β (IL-1β), interleukin 6 (IL-6), interleukin 10 (IL-10) and TNF-α in mice with chronic hypertension induced with AGII.

MATERIALS AND METHODS

Plant Materials and Extraction Both species were identified in the National Institute of Anthropology and History (INAH; Instituto Nacional de Antropología e Historia). The registration number for *O. basilicum* was 2054, and that for *O. selloi* was 2053.

Aerial parts of *O. basilicum* and *O. selloi* were shade-dried, powdered, and extracted by ethanol maceration at 60% for 72 h. The extracts of the both species were concentrated under reduced pressure (Laborota 4000, Heidolph WB eco) at 50°C. From the hydroalcoholic extracts (Oba-EtOH and Ose-EtOH), a separation was carried out in a liquid/liquid interface with ethyl acetate/water, recovering the organic part (Oba-EtOAc and Ose-EtOAc).

Standardization of Organic Extracts in Rosmarinic Acid The extracts were monitored by HPLC under the following conditions: using a Waters 2695 HPLC chromatograph equipped with a Waters 996 diode array detector module, utilizing an Empower Pro Software Program (Waters Corporation, U.S.A.), and by employing a Supelcosil LC-F column (4.6 × 250 mm i.d., 5-μm particle size) (Sigma-Aldrich, Bellefonte, PA, U.S.A.). The mobile phase consisted of an aqueous solution of 0.5% trifluoroacetic acid (solvent A) and acetonitrile (solvent B), while the gradient system was formed in the following manner: 0–1 min, 100% B; 2–3 min 5% B; 4–20 min, 30% B; 21–23 min, 50% B; 24–25 min, 80% B; 26–27 min, 100% B, and 28–30 min, 0% B. The flow was maintained at 0.9 mL/min and the injection volume was 10 μL. Absorbance was measured at 330 nm. Rosmarinic acid was identified by comparison of the retention time and the UV spectra with a commercial reference (purity grade 96%, Sigma-Aldrich). Molecular mass was corroborated by ultra-performance liquid chromatography-tandem mass spectrometer (UPLC-MS) analysis. The quantity of this polyphenolic compound in each extract was estimated by peak-area interpolation and comparison with a calibration curve of a rosmarinic acid commercial reference (Sigma-Aldrich), which was carried out using five ascendant concentrations (0.039, 0.078. 0.152, 0.3.12, and 0.624 mg/mL) injected by triplicate in the same HPLC method. This analytical process allowed to obtain a linear equation ($Y = 31874x + 134853$, $R2 = 0.9994$). Results are expressed as mean values in mg/g dry extract.

Animals and Experimental Groups Male ICR mice (age = 10 weeks; $n = 72$), under controlled conditions in a light/dark cycle (12/12 h), at a temperature of 20 ± 2°C, with free access to a special diet for rodents (Labdiet) and purified water. For the management and care of the laboratory animals during the experimentation period, we followed Official Mexican Regulation NOM 062-ZOO-1999. The research was conducted in accordance with the international accepted principles for laboratory animal use and care as found in the National Institutes of Health (NIH) guidelines. The behavioral assays were conducted in a soundproofed room with a video recording system; the experimenter avoided the use of a scent and remained in silence. The project was evaluated and authorized by the Committee of Ethics and Institutional Biosecurity and registered with number R-2016-1702-2.

Mice were randomly grouped and a blood pressure measurement was performed by a non-invasive method (LE5002 storage pressure meter; LSI Letica Scientific Instruments), determining baseline systolic pressure and baseline diastolic pressure. After that two groups were formed: A, 66 mice were administered intraperitoneally (i.p.) with AGII at 0.2 μg/kg, for six weeks; and group B with 6 mice, were not manipulated i.p. (untreated). Once the 6 weeks of the treatment with AGII were completed, the systolic blood pressure (SBP) and diastolic blood pressure (DBP) of both groups were measured again. Group A was divided into 11 sub-groups that were treated with the drugs listed in Table 1. The treatments were administered orally (*per os* (*p.o.*)) for 6 weeks and the administration of AGII was continued. After 12 weeks, blood pressure was

Table 1. Drugs Used in the Chronic Systemic Hypertension Model Induced with AGII 0.2 μg/kg

Group	Treatment (mg/kg) *p.o.*	Clave	Administration i.p. (AGII 0.2 μg/kg)
Untreated	—	Untreated	—
Negative control	Vehicle (Tween 1%)	Veh	+
AGII Receptor blocker	Telmisartan (10)	Tel 10	+
Nootropic	Galantamine (0.20)	Gal 0.2	+
Anxiolytic	Diazepam (0.25)	Dzp 0.25	+
Experimental	Organic extract of *O. basilicum* (25)	Oba-EtOAc 25	+
	Organic extract of *O. selloi* (25)	Ose-EtOAc 25	+
	Rosmarinic acid (0.39)	RA 0.39	+
	Rosmarinic acid (0.78)	RA 0.78	+
	Rosmarinic acid (1.56)	RA 1.56	+
	Rosmarinic acid (3.12)	RA 3.12	+
	Rosmarinic acid (6.24)	RA 6.24	+

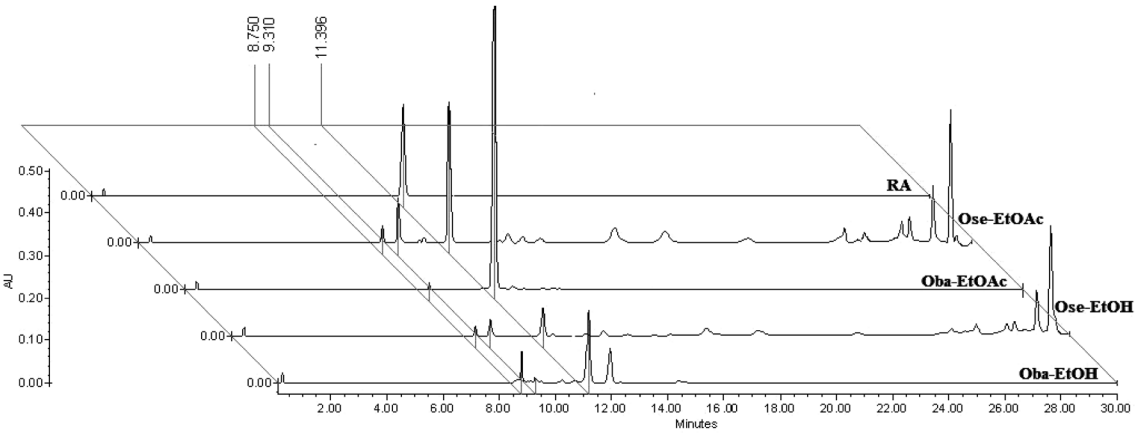

Fig. 1. Chromatographic Profile of the Ethanolic Extracts of *O. basilicum* and *O. selloi* (Oba-EtOH and Ose-EtOH), Oba-EtOAc and Ose-EtOAc, and RA Standard

The following compounds were identified and their corresponding retention times were recorded as follows: chlorogenic acid (8.7 min); caffeic acid (9.3 min), and rosmarinic acid (11.4 min). The sample of each extract was 3 mg/mL, except for that of Oba-EtOAc, which was applied at 0.75 mg/mL.

measured, the behavioral test was performed, and biological samples were taken for the biochemical and immunological tests.

Procedures At the end of the administration of AGII for 12 weeks, behavioral tests were performed. From each group, six animals were taken for the water Morris maze (WMM) and the elevated plus maze (EPM), but not for the open field test (OFT), in that all of the animals were exposed to the latter. On the experimental day, the mice were administered 60 min before the test. After each test, the mice were returned to their cages.

WMM

The WMM was first established by neuroscientist Richard G. Morris in 1981 to test hippocampal-dependent learning, including the acquisition of spatial memory and long-term memory. At week 11 of the hypertension model, training began, in where the maze was divided into four equal quadrants and a white platform was submerged 1 cm below the surface of water. The water was rendered opaque by adding a non-toxic white dye. For 5 d, each mouse was given four trainings to locate the hidden platform. During training sessions, if the animal did not find the platform in 60 s (maximum), it was gently guided to the platform and remained there for 30 s. The mouse was placed in the water each time with a starting point varying in a pseudo-random manner. They were allowed to rest for 2 d, and on day 8, the test was performed, for which the platform was removed and the time taken to go toward the platform area (latency) and the number of crossings over this area were recorded. The test was recorded on a video on the day of the test and analyzed using Smart V3.0 software.

EPM

The EPM is a behavioral trial in rodents that widely used and validated to evaluate the anxiolytic effects of drugs, based on the innate aversion of rodent to open and high spaces. It was performed according to Komada *et al.*[20] and Chioca *et al.*[21] The EPM apparatus was made of plexiglas and consisted of two open arms (30×5 cm) and two closed arms ($30 \times 5 \times 15$ cm) with 0.25-cm-thick walls. The arms extended from a central platform ($5 \text{ cm} \times 5$ cm), and the maze was elevated 50 cm from the floor of the room. Each animal was placed at the center of the maze facing one of the enclosed arms. Number of entries and time spent on closed and open arms were recorded for 5 min. An entry onto an arm was defined as the animal placing all four paws on the arm. All tests were recorded with a video camera. After each test, the maze was carefully cleaned with wet tissue paper with a 10-% ethanol solution. The percentage of number of entries onto open arms (EOA) and the percentage of time spent on open arms (TOA) were registered.

OFT

The animal's natural tendency to explode a new environment is observed, despite the fear caused by that new environment. Developed in 1934 by C. Hall, the OFT is one of the most used instruments to study anxiety, sedation, or motor activity. The OFT was carried out according to Mineur *et al.*[22] and Tatem *et al.*[23] The mice were individually placed in the center of an observation cage made of acrylic (30×30 cm), with transparent walls (15 cm) and a black floor with nine divisions marked white. The animals were observed for 5 min directly by an observer. The number of mouse rearings (standing upright on hind legs while the forepaws are free) and total crossings was registered manually.

Enzyme-Linked Immunosorbent Assay (ELISA) At the end of the behavioral test, the animals were anesthetized and blood samples were collected from retro-orbital venous plexus, centrifuged for 7 min (2200 g) and frozen at −80°C until their use. The organs (brain, right and left kidneys) were macerated with phosphate buffered saline-phenylmethylsulfonyl fluoride (0.1%) 1 : 5 w/v. The suspensions were centrifuged, and the supernatants were recovered and frozen at −80°C until their use. To perform the quantification by means of the ELISA technique of the different molecules, the inserted instructions found in each kit were followed. The biological samples utilized were brain, and right and left kidney. Serum was used for the quantification of corticosterone. Mouse IL-1β, IL-4, IL-6, IL-10, TNF-α, and MCP-1 ELISA kits were purchased from OptEIA™ BD, and Corticosterone ELISA kits were purchased from Cayman Chemical.

Statistical Analysis The results were analyzed with one-way ANOVA, applying a Tukey (post-test) for the results of measurements of blood pressure, the EPM, and the ELISA tests; and of the Dunnett (post-test) for the MWM and the OFT with a level of significance of $p < 0.05$. For all statistical analyses, the SPSS ver. 20 statistical software program was

used.

RESULTS

We analyzed all extracts of the *Ocimum* species by HPLC for detection and quantification of the major constituents of the active extract. Figure 1 presents the HPLC profile of Oba-EtOH, Ose-EtOH, Oba-EtOAc, Ose-EtOAc, and RA (standard), recorded at 330 nm. In the Oba-EtOAc extract, the presence of the major peak at a retention time of 11.396 corresponded to RA.

In both species there are similar compounds, such as chlorogenic acid (t_R: 8.7; λ 242, 325 nm) and caffeic acid (t_R: 9.3; λ 243, 325 nm). Ose-EtOAc exhibited, in 20–30 min, a compound with UV spectra for chlorophylls (Fig. 1).

Chemical Characterization of the Extracts of *O. basilicum* and *O. selloi* The quantity of this polyphenolic compound in each extract was estimated by peak-area interpolation and by comparison with a calibration curve of the RA commercial reference (Sigma-Aldrich). The amounts of RA in the Oba-EtOAc and Ose-EtOAc determined by HPLC are depicted in Table 2.

Blood Pressure Chronic administration of AGII at 0.2 μg/kg i.p. increased, by about 20 mmHg, the SBP and DBP at week 6. The increase in both pressures was maintained until week 12, which was the time at which the pharmacological test was concluded. When comparing the blood pressure results of all of the treatment groups, it was observed that there was a significant difference of the values of SBP and DBP ($*p < 0.001$; $F = 1.91$) between the damaged group (Veh) with that of the healthy animals and the values of the other treatments, including telmisartan (10 mg/kg), Oba-EtOAc (25 mg/kg), and Ose-EtOAc (25 mg/kg) (Fig. 2A) and all doses of RA (0.39–6.24 mg/kg) (Fig. 2B).

Morris Water Maze In the device that assesses the spatial learning of rodents, it was observed that the damage group or the disease model (Veh) to which AGII was administered i.p. presented a significant increase in latency time of arrival at the platform in the group compared with the group of healthy mice ($*p < 0.05$; $F = 1.928$). All of the groups of mice that received the different treatments showed a statistically significant decrease in this parameter as follows: telmisartan ($*p < 0.001$); galantamine ($*p < 0.003$); diazepam ($*p < 0.001$, and the experimental treatment ($*p < 0.003$), and Ose-EtOAc ($*p < 0.001$) (Fig. 3A). The fact that the treatment with Ose-EtOAc (25 mg/kg) had the highest nootropic effect, followed by the treatment with galantamine (0.2 mg/kg) was very noteworthy. Another parameter included the time during which the rodents kept swimming on the platform when it was submerged, therefore the number of crossings on this platform. An increase of this latter parameter was observed, which was proportional to the decrease of latency time or arrival time at the platform, as previously described (Fig. 3C), which was significantly lower when this compared with the result of the damage group ($*p < 0.021$; $F = 1.95$). The dose–response curve of RA and latency time of arrival at the platform demonstrated a significant decrease of this time as follows: 0.39 mg/kg ($*p < 0.001$); 0.78 mg/kg ($*p < 0.001$): 1.52 mg/kg ($*p < 0.004$), and 3.12 mg/kg ($*p < 0.001$) (Fig. 3B), although an inverse dose-dependent trend was identified. On the other hand, on increasing the dose, a tendency toward a decrease was observed in the number of crossings on the platform in comparison to those of the Veh (Fig. 3D).

EPM The EPM test allows to assess the degree of anxiety that rodents present, based on the behavior of the animals in the device. The chronic administration of AGII gave rise to a state of anxiety associated with hypertension (Fig. 2) and decreased learning capacity (Fig. 3). The state of anxiety associated with the administration of AGII was evidenced in the mice, due to the significant decrease in the length-of-stay TOA and the number of EOA ($*p < 0.001$; $F = 1.95$). This be-

Table 2. Standardization of Oba-EtOAc and Ose-EtOAc Extracts, in Rosmarinic Acid

Species	RA (mg/g of extract)	Dosage of RA administered in animal model
Ocimum basilicum	251.65	6.29 mg/kg
Ocimum selloi	15.95	0.39 mg/kg

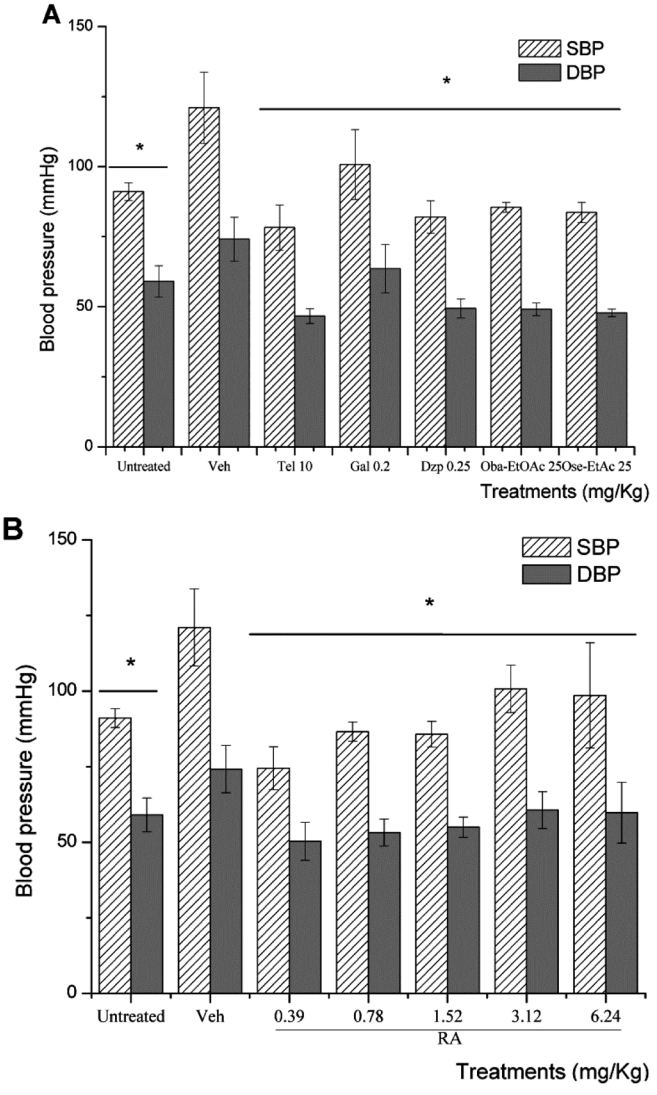

Fig. 2. Effect of the Different Treatments on Blood Pressure in the Chronic Hypertension Model

$*p < 0.001$; $F = 1.91$ compared to the Vehicle (Veh) with analysis by ANOVA with the Tukey post-test. The value represents the mean ± standard deviation (S.D.) ($n = 10$). SBP and DBP. Oba-EtOAc, Ose-EtOAc, Tel, telmisartan; Gal, galantamine; Dzp, diazepam, and RA (0.39–6.24 mg/kg).

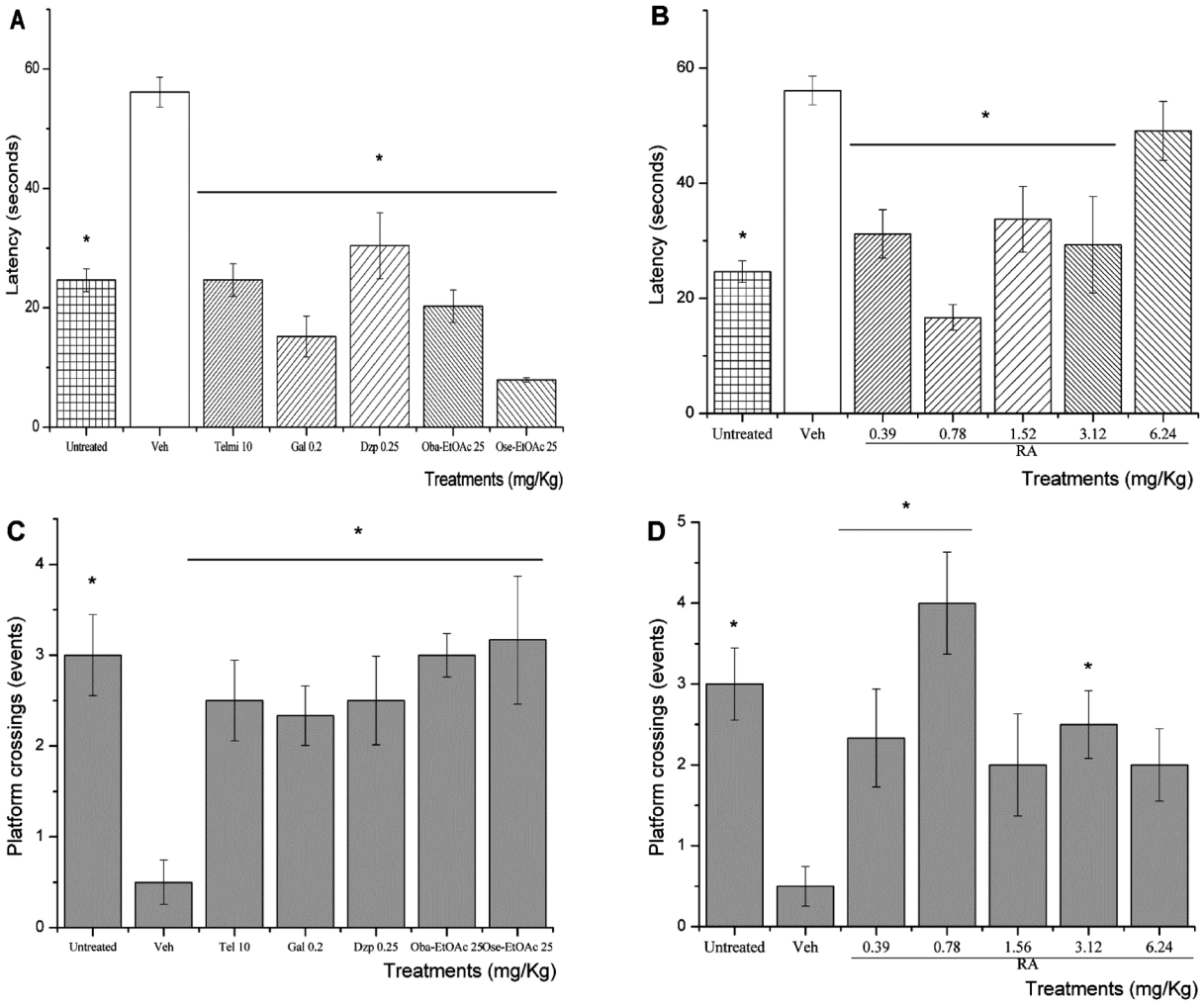

Fig. 3. Effect of (A, C) Control Groups and Oba-EtOAc (25 mg/kg) and Ose-EtOAc (25 mg/kg), and Different Doses of RA (B, D) in the Water Morris Maze

*$p < 0.001$; $F = 1.91$ compared to the vehicle (Veh), with analysis by ANOVA with the Dunnett post-test. The value represents the mean ± S.D. ($n = 6$). Tel, telmisartan; Gal, galantamine; Dzp, diazepam.

havior was reversed both in the treatment groups with control drugs, and in the experimental treatment groups (Oba-EtOAc and Ose-EtOAc), and in the same manner with all of the tested doses of RA (Figs. 4A, B). From the dose–effect curve of RA as anxiolytic, the following pharmacological constants were established: EOA: $E_{max} = 63.69\%$; $ED_{50} = 0.025$ mg/kg; TOA: $E_{max} = 59.17\%$, and

OFT With the intention of consolidating the behavioral results of the nootropic and anxiolytic effect of the experimental treatments, the spontaneous motility of the animals exposed to the administration Oba-EtOAc (25 mg/kg) and Ose-EtOAc (25 mg/kg) was evaluated. The motility assessment was performed in an open-field device, in which it was established that the chronic administration of AGII caused the significant decrease in motility evaluated by the measurement of the total crossings and vertical surveys (rearings) and corresponded to 51 and 35%, respectively, compared with those of the untreated group, which could suggest that the anxiolytic effect would have to be reconsidered. However, the decrease in motility time was determined by the time consumed by the animals in grooming, which is also a parameter indicative of the state of anxiety, in addition, the increase in the number of excreta observed (data not shown). Treatments for the administration of galantamine, diazepam, Ose-EtOAc, and the first four doses of RA revealed significant increases in total crossings compared with those of the damage group (*$p < 0.001$) (Table 3). From the dose–effect curves of RA, the following pharmacological constants were calculated: $E_{max} = 204.08$ events; $ED_{50} = 0.081$ mg/kg for total crossings, and $E_{max} = 61.35$, $ED_{50} = 0.006$ mg/kg for rearings.

ELISA

Corticosterone

Sustained stimulation of the HPA axis gives rise to a state of anxiety associated with increased serum corticosterone levels. In the proposed hypertension model, chronic administration of AGII caused the condition of behavioral anxiety (Fig. 4), parallel to the significant increase in the serum corticosterone concentration by approximately 25% (*$p < 0.05$; $F = 1.95$). Administration of the control treatments and of the experimental treatments reversed the effect observed in the damage group (Fig. 5A). Similarly, in the dose-response curve of RA, dose-dependent corticosterone-level behavior was observed, which corresponds to the observed behavioral anxiolytic effect (Fig. 4). This behavior was observed between the 0.39 and the 3.12 mg/kg dose.

Inflammatory Damage Markers: TNF-α, IL-1β, IL-6,

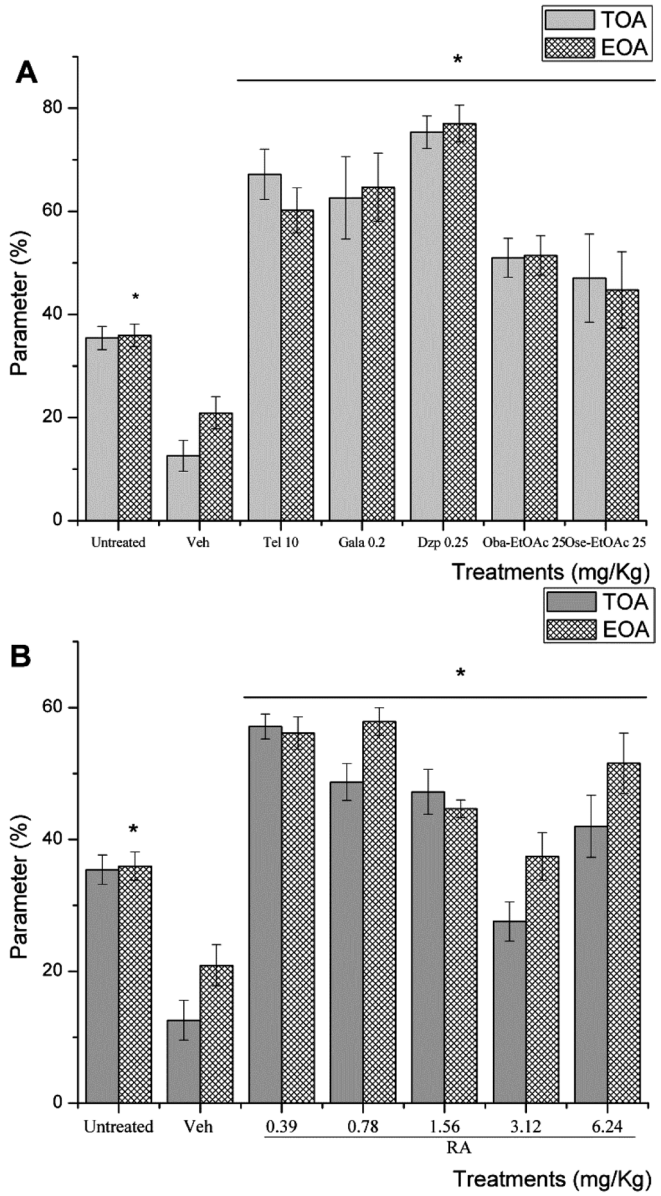

Fig. 4. Anxiolytic Effect of the Treatment with the Oba-EtOAc (25 mg/kg) and Ose-EtOAc (25 mg/kg) on the Anxiety Caused by the Chronic Administration of AGII

$*p < 0.05$; $F = 1.95$ in comparison with vehicle (Veh) by ANOVA and the Tukey post-test. Values are means ± standard error of mean (S.E.M.) ($n = 6$). TOA: time spent on open arms; EOA: entries onto open arms: Untreated, without the administration of AGII; Veh, vehicle; Tel, telmisartan; Gal, galantamine; Dzp, diazepam; Oba-EtOAc; Ose-EtOAc, and RA (0.39–6.24 mg/kg).

MCP-1, and IL-10

Table 4 presents the tissue concentrations of cytokines in organs (brain, and right and left kidneys) that respond to vascular damage, caused by arterial hypertension secondary to chronic administration of AGII. In the damage group, a significant increase was observed in the concentrations of TNF-α, IL-1β, and IL-6 (cytokines considered as proinflammatory), both in the brain and in both kidneys ($*p < 0.05$; $F = 1.91$).

A decrease in the level of TNF-α in brain and left kidney is observed with the administration of telmisartan, galantamine, diazepam, and the extracts of both Oba-EtOAc and Ose-EtOAc ($*p < 0.001$).

IL-1β decreases in brain and right kidney with the administration of telmisartan, galantamine, Oba-EtOAc, and Ose-EtOAc, while it decreases in the left kidney with the administration of telmisartan and Ose-EtOAc ($*p < 0.001$).

Table 3. Effect on Spontaneous Motility in the OFT Device, of the Oba-EtOAc and Ose-EtOAc Extracts, on the Motility Modification Caused by the Chronic Administration of AGII

Group	Total crosses	Rearing
Untreated	139.6 ± 16.10*	37 ± 11.39*
Veh	69.33 ± 15.66	24.55 ± 4.69
Tel 10	81.42 ± 7.61	27.28 ± 6.96
Gal 0.2	105.33 ± 14.26*	45.66 ± 7.95*
Dzp 0.25	97.37 ± 17.22*	28.12 ± 11.37
Oba-EtOAc 25	75.33 ± 8.97	27.11 ± 5.19
Ose-EtOAc 25	107.28 ± 10.64*	42.14 ± 5.08*
RA 0.39	121.6 ± 11.17*	43.2 ± 3.70*
RA 0.78	107.2 ± 17.54*	41.8 ± 6.37*
RA 1.56	89.4 ± 16.83*	33 ± 13.76
RA 3.12	88.2 ± 11.84*	20 ± 6.04
RA 6.24	79.2 ± 12.49	19 ± 5.43

Untreated, without administration of AGII; Veh, vehicle; Tel, telmisartan; Gal, galantamine; Dzp, diazepam; Oba-EtOAc, Ose-EtOAc and RA (0.39–6.24 mg/kg). Compared to the Vehicle by ANOVA post-test Dunnet ($n = 6$).

The concentration of IL-6 in the brain is decreased by the administration of telmisartan, galantamine, and the Ose-EtOAc extract ($*p < 0.001$). In the right kidney, the concentration of IL-6 is decreased compared to that of the Veh group with the administration of telmisartan, galantamine, and Ose-EtOAc, while in the left kidney, by means of the administration of telmisartan, galantamine, diazepam ($*p < 0.014$), and Ose-EtOAc ($*p < 0.001$).

There was no significant difference between the concentrations of IL-10 in brain and both kidneys of the untreated and Veh groups. However, in the brain, galantamine ($*p < 0.006$) and diazepam ($*p < 0.004$), in the right kidney, galantamine ($*p < 0.002$) and Oba-EtOAc ($*p < 0.003$), and in the left kidney, telmisartan ($*p < 0.001$), galantamine ($*p < 0.001$), diazepam ($*p < 0.001$), Oba-EtOAc ($*p < 0.001$), and Ose-EtOAc ($*p < 0.002$) significantly increased the level of IL-10 compared to that of the Veh group.

The chronic administration of AGII also increased the tissue concentration of MCP-1, which gave rise to a decrease in the permeability of the endothelium in brain and both kidneys, on comparison with that of the untreated group (healthy) ($*p < 0.001$). The administration of the treatments significantly decreased the level of this chemokine when compared with the damage group. telmisartan (both kidneys; $*p < 0.001$), galantamine (brain; $*p < 0.011$), left kidney; $*p < 0.001$), diazepam (both kidneys, $*p < 0.001$); Oba-EtOAc (brain, $*p < 0.036$; both kidneys, $*p < 0.001$), and Ose-EtOAc (brain, $*p < 0.001$; both kidneys; $*p < 0.001$).

With respect to the RA curve (Table 5), all doses administered (0.39, 0.78, 1.52, 3.12, and 6.24 mg/kg) decreased the concentrations of TNF-α, IL-1β, IL-6, and MCP-1 and increased the concentrations of IL-10 and IL-4 with regard to the Veh group ($*p < 0.05$). In these cytokines, reverse dose-dependent behavior was observed, that is, the lower the concentration, the greater the modulator effect.

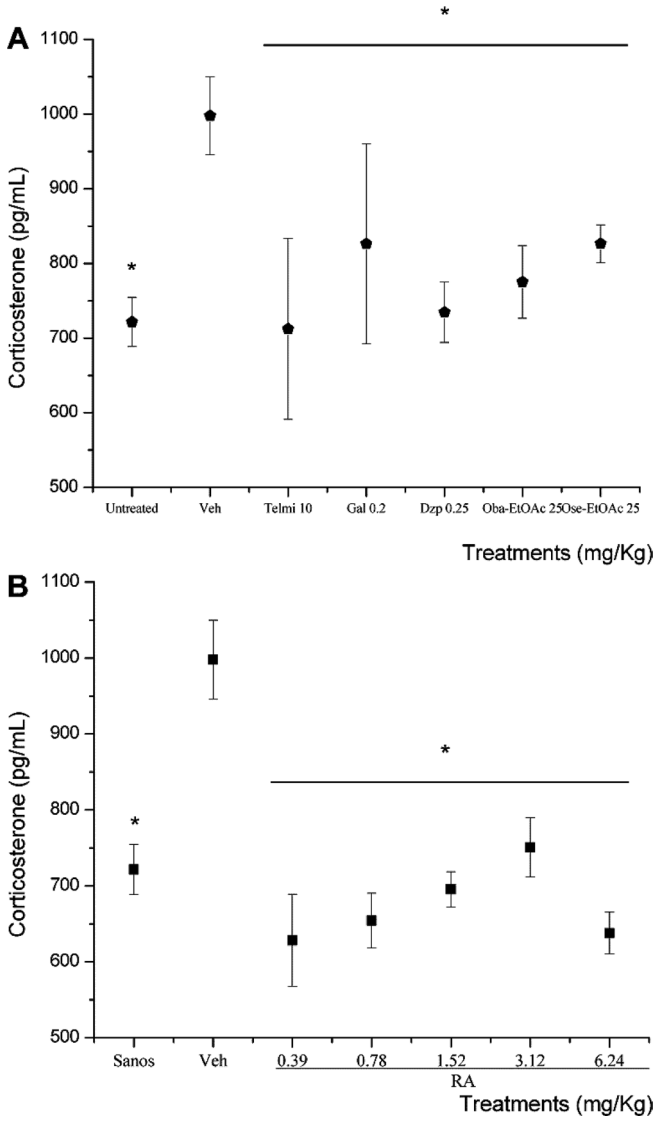

Fig. 5. Antagonistic Effect of the Treatments with Extracts Oba-EtOAc and Ose-EtOAc on the Release of Corticosterone Caused by the Chronic Administration of AGII

The values presented correspond to the serum concentration ± S.D. ($n = 6$). Untreated, without administration of AGII; Veh, vehicle; Tel, telmisartan; Gal, galantamine; Dzp, diazepam; Oba-EtOAc; Ose-EtOAc and RA (0.39–6.24 mg/kg). *$p < 0.05$; $F = 1.95$ in comparison with Vehicle by ANOVA post-test Tuckey.

DISCUSSION

The results of the present work indicate that the ethyl acetate extracts of *O. basilicum* and *O. selloi* regulate some of the damage responses generated by chronic AH, which was generated in the mice by the administration during 12 weeks of AGII.

These plants share the presence of caffeic acid, chlorogenic acid, and RA. Chemical analysis allowed observing that, for *O. basilicum*, RA is the majority compound (251.65 mg RA/gram of extract), while, for *O. selloi*, the content of RA was 15.95 mg/gram of extract. Based on this, both extracts were standardized in terms of their RA content, and the evaluation of this compound was carried out at different doses (0.39, 0.78, 1.52, 3.12, and 6.24 mg/kg) in the chronic AH assay.

In the present report, the chronic administration was employed of AGII for the induction of damage associated with chronic AH, due to that it has been shown that the infusion of this hormone induces an elevation in SBP.[5,24] Hypertension possesses a multifactorial etiology in which genetic, psychosocial, and environmental factors appear to be important.[9] Hypertension injures blood vessels and thereby causes end-organ damage. The endothelial layer acts as a signal-transduction interface for hemodynamic forces in the regulation of vascular tone and the chronic structural remodeling of arteries.[6] Consequently, the mechanisms of regulation mediated by AGII have become a therapeutic target in the treatment of AH and its metabolic complications.[6,25] After 6 weeks of daily administration of AGII, treatment with the extracts or with RA was initiated; the result of this was that all treatments decreased hypertension, even to the degree of comparison with animals that did not receive AGII (untreated).

For *O. basillicum*, there is experimental evidence that contributes to the knowledge of how this plant species may be exerting its protective effect against the actions of AGII. It was shown that it is capable of strongly protecting the myocardium against isoproterenol-induced infarction and suggests that the cardioprotective effects could be related to antioxidative activities.[26] Umar *et al.* report that the crude extract of *O. basilicum* (100–400 mg/kg) decreased the blood pressure (BP) level in rat in a dose-dependent manner.[27] It was also reported to cause a vasorelaxant effect in rat aortic rings, though the mechanism for this relaxation was not determined.[28] Perhaps one potential action mode could be due to sweet basil's potent ROS scavenging ability.[29,30]

In the case of *O. selloi*, there is no evidence, to our knowledge, to indicate its pharmacological activity on hypertension and its consequences, even though, in traditional medicine in Mexico, one of the main medicinal uses is against hypertension. While it is necessary to delve more into this species and its benefits, in this work we showed that the extract of both plants are capable of diminishing the hypertension generated by AGII.

This may be partly attributable to its RA content, since this substance is capable of inhibiting the angiotensin-converting enzyme (ACE),[31,32] or it may exert a vasodilatory effect that is endothelium-dependent.[33] It has been shown that RA protects the brain against damage caused by ischemia–reperfusion by maintaining the integrity of the blood–brain barrier.[34] However, in the organic extract of *O. selloi*, RA is found at a lower concentration as compared to *O. basillicum*; thus, the vasoactive activity of the extract is probably due to interaction with other compounds.

The effect of the extracts and of the RA were similar to those observed with telmisartan, which is an antagonist of the receptors to angiotensin type 1 (rAT1), and it is probable that *O. selloi* and *O. basilicum* also contain compounds that act in a manner similar to that of the drug. Nonetheless, it should be mentioned that this differentiation toward a regulating phenotype of monocytes may be independent of the rAT1 pathway and/or the antagonism of peroxisome proliferator-activated receptor gamma (PPARγ).[35]

It has been described that there is a comorbidity between AH and psychiatric disorders such as anxiety, depression, and cognitive deterioration processes, among others.[9,36,37] The existence of such associations would be consistent with work indicating that such symptoms are accompanied by alterations in peripheral and central neuro-endocrine systems.[36] In par-

Table 4. Effect of the Treatments Oba-EtOAc and Ose-EtOAc on the Tissue Levels of Cytokines of Widely Vascularized Organs

Organ	Treatment (mg/kg)	TNF-α (pg/mg prot)	IL-1β (pg/mg prot)	IL-6 (pg/mg prot)	IL-10 (pg/mg prot)	IL-4 (pg/mg prot)	MCP-1 (pg/mg prot)
Brain	Untreated	110.70 ± 39.60*	510.94 ± 47.25*	62.29 ± 16.20*	84.96 ± 18.50	134.33 ± 22.15*	41.28 ± 5.65*
	Vehicle	588.69 ± 65.36	697.38 ± 108.78	214.51 ± 83.80	81.92 ± 24.33	199.27 ± 54.92	99.73 ± 31.78
	Tel 10	302.28 ± 91.46*	570.23 ± 65.62*	86.12 ± 11.34*	63.97 ± 7.98	93.26 ± 13.68*	73.27 ± 31.66
	Gal 0.2	231.10 ± 38.06*	464.41 ± 104.71*	85.50 ± 9.01*	43.03 ± 13.26*	73.17 ± 9.00*	62.97 ± 12.75*
	DZP 0.25	214.34 ± 16.17*	447.32 ± 70.13*	180.85 ± 12.77	42.33 ± 5.85*	73.42 ± 10.84*	76.44 ± 9.37
	Oba-EtOAc 25	293.63 ± 53.78*	500.30 ± 67.88*	201.14 ± 28.64	57.85 ± 4.50	87.05 ± 10.46*	66.72 ± 23.18*
	Ose-EtOAc 25	285.19 ± 62.41*	355.47 ± 29.90*	104.52 ± 29.94*	62.12 ± 5.56	153.79 ± 30.92*	55.22 ± 7.65*
Right kidney	Untreated	114.53 ± 49.71*	556.93 ± 98.25*	217.41 ± 86.41*	113.73 ± 22.74	345.25 ± 44.51	82.57 ± 8.27*
	Vehicle	1955.73 ± 395.75	3654.68 ± 468.84	1251.75 ± 176.73	179.10 ± 72.65	303.10 ± 38.33	185.37 ± 42.32
	Tel 10	492.21 ± 160.43*	492.21 ± 160.43*	375.30 ± 118.09*	191.22 ± 68.30	419.21 ± 76.53*	127.87 ± 14.66*
	Gal 0.2	2227.73 ± 246.44	2227.73 ± 246.44*	723.06 ± 256.47*	281.99 ± 48.95*	464.60 ± 43.07*	154.15 ± 42.58
	DZP 0.25	1984.68 ± 168.63	5029.35 ± 439.36*	1070.73 ± 374.11	190.88 ± 53.73	458.16 ± 45.86*	125.05 ± 16.06*
	Oba-EtOAc 25	1191.30 ± 85.65*	3003.22 ± 238.41*	1015.78 ± 147.90	157.91 ± 41.65	410.02 ± 60.08*	111.64 ± 29.57*
	Ose-EtOAc 25	1416.37 ± 167.23*	1416.37 ± 167.23*	653.62 ± 91.79*	278.99 ± 73.09*	541.10 ± 79.78*	72.85 ± 16.16*
Left kidney	Untreated	262.73 ± 43.73*	1829.48 ± 122.55*	171.84 ± 33.62*	71.40 ± 15.96	254.29 ± 63.19	82.74 ± 16.72*
	Vehicle	2081.06 ± 301.41	3753.63 ± 702.05	1703.19 ± 85.83	92.47 ± 25.69	282.32 ± 29.03	323.92 ± 52.04
	Tel 10	570.43 ± 90.06*	2664.81 ± 377.45*	492.62 ± 171.59*	292.32 ± 46.99*	490.49 ± 72.66*	95.29 ± 13.17*
	Gal 0.2	1710.29 ± 176.82*	4058.40 ± 417.84	676.64 ± 55.69*	273.95 ± 48.43*	408.12 ± 39.56	155.23 ± 17.28*
	DZP 0.25	1327.03 ± 167.76*	3924.26 ± 242.17	1430.43 ± 201.89*	227.52 ± 47.50*	455.42 ± 46.06*	177.44 ± 34.86*
	Oba-EtOAc 25	1807.78 ± 195.49*	3653.14 ± 432.73	1610.02 ± 239.06	348.92 ± 105.27*	609.21 ± 174.57*	94.92 ± 30.05*
	Ose-EtOAc 25	1781.64 ± 262.13*	2921.56 ± 487.79*	734.12 ± 137.27*	192.98 ± 48.84*	617.41 ± 156.31*	64.37 ± 13.56*

The concentration of cytokines (TNF-α, IL-1β, IL-6, IL-10, IL-4) and chemokine (MCP-1) is expressed in pg/mg of protein modulator effect. *$p < 0.05$ ($F = 1.91$) in comparison with Vehicle by ANOVA post-test Tuckey. Values are means ± S.D. ($n = 8$). Tel, telmisartan; Gal, galantamine; Dzp, diazepam; Oba-EtOAc; Ose-EtOAc.

Table 5. Effect of the Dose Response Curve of RA on the Tissue Levels of Cytokines of Widely Vascularized Organs

Organ	Treatment (mg/kg)	TNF-α (pg/mg prot)	IL-1β (pg/mg prot)	IL-6 (pg/mg prot)	IL-10 (pg/mg prot)	IL-4 (pg/mg prot)	MCP-1 (pg/mg prot)
Brain	Untreated	110.70 ± 39.60*	510.94 ± 47.25*	62.29 ± 16.20*	84.96 ± 18.50	134.33 ± 22.15*	41.28 ± 5.65*
	Vehicle	588.69 ± 65.36	697.38 ± 108.78	214.51 ± 83.80	81.92 ± 24.33	199.27 ± 54.92	99.73 ± 31.78
	RA 0.39	156.98 ± 23.66*	366.38 ± 40.94*	139.64 ± 16.86*	58.39 ± 12.44	142.16 ± 5.37*	106.54 ± 17.36
	RA 0.78	158.92 ± 27.95*	643.40 ± 61.43	141.00 ± 19.31*	68.66 ± 2.15	200.47 ± 28.23	110.67 ± 23.39
	RA 1.52	314.48 ± 16.67*	631.57 ± 34.89	152.63 ± 8.49*	76.22 ± 7.22	111.73 ± 10.56*	91.93 ± 3.09
	RA 3.12	390.22 ± 26.26*	751.66 ± 54.02	165.50 ± 15.44	81.14 ± 10.34	104.21 ± 3.33*	89.40 ± 18.05
	RA 6.24	581.70 ± 85.10	1011.22 ± 84.84*	175.53 ± 22.14	125.10 ± 45.20*	114.26 ± 12.83*	78.28 ± 15.96
Right kidney	Untreated	114.53 ± 49.71*	556.93 ± 98.25*	217.41 ± 86.41	113.73 ± 22.74	345.25 ± 44.51	82.57 ± 8.27*
	Vehicle	1955.73 ± 395.75	3654.68 ± 468.84	1251.75 ± 176.73	179.10 ± 72.65	303.10 ± 38.33	185.37 ± 42.32
	RA 0.39	1268.18 ± 75.94*	2767.86 ± 100.92*	768.28 ± 51.84*	142.15 ± 14.83	434.16 ± 34.78*	128.55 ± 11.95*
	RA 0.78	1461.72 ± 74.45*	3276.73 ± 119.02*	860.88 ± 30.83*	184.76 ± 13.45	487.60 ± 27.09*	122.12 ± 19.95*
	RA 1.52	1645.24 ± 91.84*	3453.32 ± 245.02	983.71 ± 73.59	219.70 ± 39.54	433.70 ± 15.01*	111.70 ± 35.07*
	RA 3.12	1818.45 ± 153.33	3625.40 ± 287.50	1161.72 ± 74.38	292.45 ± 19.16*	374.53 ± 15.11	98.15 ± 9.02*
	RA 6.24	2044.74 ± 147.87	3305.71 ± 217.31	1148.60 ± 92.92	301.27 ± 26.80*	385.28 ± 43.45*	76.27 ± 22.28*
Left kidney	Untreated	262.73 ± 43.73*	1829.48 ± 122.55	171.84 ± 33.62*	71.40 ± 15.96	254.29 ± 63.19	82.74 ± 16.72*
	Vehicle	2081.06 ± 301.41	3753.63 ± 702.05	1703.19 ± 85.83	92.47 ± 25.69	282.32 ± 29.03	323.92 ± 52.04
	RA 0.39	1142.82 ± 72.12*	2306.54 ± 217.50*	927.37 ± 55.43*	270.34 ± 14.97*	403.06 ± 23.11*	249.38 ± 35.28*
	RA 0.78	1406.60 ± 96.62*	2862.79 ± 188.74*	984.02 ± 101.88*	310.11 ± 11.07*	483.24 ± 43.25	141.32 ± 13.08*
	RA 1.52	1642.00 ± 108.50*	3258.38 ± 183.20	1119.69 ± 135.22*	317.32 ± 38.20*	413.37 ± 15.22	115.70 ± 21.76*
	RA 3.12	1833.37 ± 87.26	3533.09 ± 145.19	1282.89 ± 220.74*	317.34 ± 36.14*	398.79 ± 32.94	85.47 ± 20.35*
	RA 6.24	1816.60 ± 113.02	3463.46 ± 255.71	1411.30 ± 104.60*	354.02 ± 17.62*	355.00 ± 14.59	78.09 ± 25.37*

The concentration of cytokines (TNF-α, IL-1β, IL-6, IL-10, IL-4) and chemokine (MCP-1) is expressed in pg/mg of protein. *$p < 0.05$ ($F = 1.91$) in comparison with Vehicle by ANOVA post-test Tuckey. Values are means ± S.D. ($n = 8$). RA (0.39–6.24 mg/kg).

ticular, two systems are mobilized under stress: the fast-acting sympathetic nervous system, and the slow HPA axis. Sympathetic nervous-system responses include the release of the catecholamines adrenaline and noradrenaline from the adrenal medulla. Activation of the HPA axis leads, *via* intermediate steps, to the release of glucocorticoids (mainly cortisol in humans, and of corticosterone in rodents) from the adrenal cortex.[38]

There is a role of brain AGII, and in particular, of its rAT1 in the regulation of the response to stress. rAT1 are concen-

trated throughout the HPA axis and in higher centers, controlling the response of the HPA axis to stress.[11] The resulting stimulation of the brain regions and pituitary AGII systems activates the HPA axis, enhances the formation and release of corticotropin-releasing hormone (CRH), increases the release of the pituitary adrenocorticotropic hormone (ACTH), and increases the formation and release of adrenal corticosterone.[11,39–41]

Therefore, many extracts of *O. basilicum*, *O. selloi*, and all doses of RA were able to antagonize the anxiogenic effect caused by the chronic administration of AGII within a context of arterial hypertension. The evidence was obtained by the increase of the time of permanence and the number of EOA in the EPM. The dose–response curve of the RA generated the pharmacological constants of $E_{max} = 63.69\%$; $ED_{50} = 0.23$ mg/kg, for EOA; and for TOA: $E_{max} = 59.17\%$: ED_{50} 0.15 mg/kg. The trend that reveals the behavior of the values represent an inverse U-shaped dose–response curve for anxiolytic-like effects. This dose–response curve is usually explained by the actions of drug on different systems (stimulant, depressant) with different thresholds of sensitivity to those drugs.[42]

In the published report of Pereira *et al.*, the authors administered RA doses of 1, 2, 4, and 8 mg/kg and the animals were exposed to different behavioral models, among these the following: EPM, step-down inhibitory avoidance (memory), and open field task in rats. These authors observed an increase in EOA at doses of 2 and 4 mg/kg i.p. of RA, without affecting short-term-memory and long-term-memory retention in the inhibitory avoidance task. RA at 8 mg/kg induced an increase in anxiety and locomotor activity in rats.[42] In this work, we utilized doses lower than 8 mg/kg; thus, we did not observe the anxiogenic effect of the RA in the EPM and open field tests. It is noteworthy that only the *O. selloi* extract with RA at 0.39, 0.78, 3.52, and 6.24 mg/kg counteracted the depressant effect of the locomotor system of the AGII in the OFT.

Although the majority of studies have focused on the effects of stress before learning, after learning, or before memory testing, there is recent evidence that stress can also influence subsequent memory if it is presented after retrieval, thus suggesting that stress also affects the reconsolidation and/or extinction processes.[38,43,44] Conditions with chronically elevated glucocorticoid levels are usually associated with impaired cognitive performance, and these deficits are thought to result from a cumulative and long-lasting burden on hippocampal function and morphology.[45–47]

In this work, we observed that, when administering AGII i.p. for 12 weeks, the serum concentration of corticosterone was increased to 997.69 ± 46.53 pg/mL. By contrasting these results with the assessment of learning ability in WMM, an increase in latency and a decrease in the number of crossings over the area of the platform, indicating impaired learning and/or memory, are observed.

When the experimental treatments were administered based on the extracts of *O. basilicum* and *O. selloi*, this harmful effect of AGII is counteracted, that is, latency-time arrival at the platform decreased and the number of crossings on the submerged platform increased. When the doses of 0.39, 0.78, and 3.12 of RA were administered, both parameters were modified.

In the work reported by Hasanein and Mahtaj, administration of RA at 8 mg/kg *p.o.* for 7 d, did not alter cognitive function in control and scopolamine-treated groups exposed to the passive avoidance model. The combination of anticholinesterase, neuroprotective, and antioxidant properties of RA may be responsible for the observed effects.[48] In another work, the authors suggested that RA exerts a cognitive-enhancing effect that may be mediated by the inhibition of prolyl oligopeptidase (POP), which is involved in memory-related function.[49]

Antianxiety treatment is effective in lowering blood pressure in patients with excessive hypertension.[50] The clinical use of benzodiazepines is limited by the development of tolerance to their pharmacological effects.[51]

AGII is primarily involved in the inflammatory process by modulating cytokine release and proinflammatory transcription factors such as NF-κB.[3] Inflammation is well-documented as contributing to vascular remodeling and consequent hypertension. NF-κB, a transcription factor, is known to partake in the pathology of hypertension. It induces endothelial-cell dysfunction, oxidative stress, and inflammation[52,53] through the release of proinflammatory cytokines, such as TNF-α and IL-6.[53,54]

IL-1 is one of the most critical molecules involved in neuroendocrine and neurobehavioral stress responses. Many types of stressors induce the production of IL-1, both in the periphery and within the brain. The production of stress-induced IL-1 affects many physiological and behavioral systems, and one of the primary effects of stress-induced elevation in brain IL-1 is activation of the HPA axis.

Stress-induced IL-1-mediated secretion of glucocorticoids alters various neurobehavioral processes. In particular, IL-1 plays an important role in the stress-induced modulation of memory functioning. Specifically, low levels of brain IL-1 (which may be elicited by exposure to the stress associated with aversive learning paradigms) promote memory consolidation, whereas high levels of stress-induced IL-1, particularly in chronic situations, impair memory consolidation.[46]

Inflammation plays critical role in the pathogenesis of AGII-induced heart and renal injury,[7,55] and the anti-inflammatory effects of IL-10 have been shown to play a protective role in cardiovascular disease (CVD).[5,56]

Wang *et al.* demonstrated that cytokines, such as TNF-α, IL-1β, IL-6, IL-10, interferon gamma (IFN-γ), and transforming growth factor beta (TGF-β), are highly expressed in AGII-induced hypertension.[55]

The key inflammatory molecule in initiating vascular, cardiac, and renal inflammatory responses may be MCP-1, an important mediator of the activation and recruitment of monocytes into the vascular endothelium.[6,57]

For *O. basilicum*, there are reports of its antioxidant[30] and anti-hypertensive[27] effects. Such an antioxidant effect is generally attributed to the presence of RA and caffeic acid.[30] In situations in which the possible mechanism is related to the modulation of the production of ROS, it could also be modulating the expression of NF-κB, and consequently inhibiting the production of proinflammatory cytokines and chemokines.

For the case of *O. selloi*, since the RA is at a lower concentration, the modulating activity of the inflammation could be associated with unidentified compounds of the polyphenol type,[58] and even some pigments such as chlorophylls.[59,60]

CONCLUSION

In addition to the control and prevention of arterial hypertension, the consequences of the latter continue to be one of the priority topics in the area of public health. Although there are treatments to control blood pressure, alternatives are still being sought, especially in the field of phytotherapy.

In the present study, we propose *O. basilicum* and *O. selloi*, and rosmarinic acid, which have shown an effect on the inflammatory response and its consequences in the state of response to stress.

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Conflict of Interest The authors declare no conflict of interest.

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