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BIOMEDICAL SCIENCES

The water extract and the lectin WSMoL from the seeds of *Moringa oleifera* prevent the hypertension onset by decreasing renal oxidative stress

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Abstract: Maternal endotoxemia disturbs the intrauterine environment, impairs nephrogenesis, and increases the risk of hypertension and kidney disease in adulthood. Here, it was investigated whether maternal treatment with the water extract of *Moringa oleifera* seeds (WEMoS) or the water-soluble *M. oleifera* seed lectin (WSMoL) prevents the oxidative stress induced by lipopolysaccharide (LPS) in pregnant rats, and the renal injury and hypertension in the adult offspring. The administration of WEMoS or WSMoL prevented the stimulatory effects of LPS on lipid peroxidation in the maternal-placenta-fetuses environment. The impact of WEMoS was linked to decreased superoxide anions production in the placenta. The effects of WSMoL were parallel to the inhibition of superoxide anion production and NADPH oxidase activity. The WSMoL also prevented increased NADPH oxidase activity in the fetal kidney. The LPS offspring presented higher systolic blood pressure (SBP) and increased lipid peroxidation, reactive oxygen species (ROS), NADPH oxidase activity, and nitrate/nitrite in the kidney; the maternal treatment with WEMoS and WSMoL prevented these changes. In conclusion, the present study demonstrates that WEMoS and WSMoL have protective effects on maternal endotoxemia, which involve antioxidant and anti-inflammatory actions that prevent the programming of hypertension.

Key words: Hypertension, lectin, *Moringa oleifera*, reactive oxygen species.

INTRODUCTION

Adverse intrauterine events increase the risk of chronic diseases in postnatal life (Nüsken et al. 2018). This phenomenon is known as the Developmental Origin of Adult Diseases, and oxidative stress presents a pivotal role in fetal growth retardation as well as is crucial in injuries and dysfunction of several tissues after birth, reinforcing its position linking intrauterine insult and the programming of diseases (Hsu & Tain 2020). Maternal endotoxemia is related to the delay of intrauterine development and adulthood hypertension programming, which occurs dependently on the elevation of intrauterine oxidative stress (Vieira et al. 2018, Farias et al. 2020).

Blood pressure regulation occurs mainly through handling plasma volume, cardiac output, and vascular resistance. It has already been demonstrated that maternal exposure to

lipopolysaccharide (LPS) impairs nephrogenesis and leads to renal structural damage (Hao et al. 2010, Wang et al. 2014) and morphological abnormalities of the myocardium and aorta in adulthood (Zhao et al. 2014, Wang et al. 2015, Chen et al. 2015). These changes may lead to inadequate blood pressure control and are partially dependent on oxidative stress (Vieira et al. 2018). The antioxidant substances, such as alpha-tocopherol, are potentially able to prevent the elevation of blood pressure induced by maternal adverse events; however, the maternal antioxidant treatment may also cause nephrogenesis impairment and hypertension in adult life when administered under control conditions (Vieira-Filho et al. 2014). Considering the role of reactive oxygen species (ROS) in the programming of hypertension and the adverse effects of unspecific antioxidant treatment, it is fundamental to identify antioxidant substances without negative consequences on nephrogenesis.

Moringa oleifera is a tree species native to India and is widely cultivated in Africa, Central and South America, and Asia (Ayerza 2012). Several parts of the plant have multiple applications in ethnopharmacology. The leaves and seeds are rich in polyphenols, showing several beneficial effects, including anti-inflammatory, anticancer, and antimicrobial properties (Muangnoi et al. 2012, Pontual et al. 2014). Among the beneficial effects of *M. oleifera*, it must be highlighted the antioxidant capacity described in several conditions for the leaves (Duranti et al. 2021a,b, Ceci et al. 2022) and seeds (Gupta et al. 2007, Mishra et al. 2009, Velaga et al. 2014, Obembe 2019). In addition to exhibiting nutritional and medicinal values (Ogbunugafor et al. 2011, Farooq et al. 2012), their seeds contain water-soluble proteins useful for treating water (Bhuptawat et al. 2007, Lea 2010, Vasconcelos & Oliveira 2004). It has already been described that the extract of the leaves of *M. oleifera* has renoprotective effects (Oguntibeju et al. 2020). However, no data exists on the effects of extracts from the seeds. It is known that the lectin WSMoL (water-soluble *M. oleifera* lectin), isolated from the *M. oleifera* seeds (Coelho et al. 2009), presents antioxidant, immunomodulatory and anti-inflammatory activities (Santos et al. 2005, Araújo et al. 2013, Coriolano et al. 2018). This lectin is characterized as a 60 kDa molecule (De Moura et al. 2016). It is recognized as partially accountable for the water-clarifying effect of the seeds (Ferreira et al. 2011, Freitas et al. 2016).

Considering the impact of oxidative stress on the fetal development and the programming of hypertension, the water extract of *M. oleifera* seeds (WEMoS) and the WSMoL may be helpful in the management of intrauterine adverse conditions. Therefore, the present study aimed to test whether maternal WEMoS and WSMoL administration would prevent the elevation of blood pressure induced by maternal exposure to LPS and to evaluate whether this protection would depend on antioxidative effects in dams, fetuses, and adult offspring.

MATERIALS AND METHODS

Materials

Escherichia coli lipopolysaccharide (0111: B4), tempol, Folin-Ciocalteau reagent, bovine serum albumin, malondialdehyde, 2-thiobarbituric acid (TBA), 5,5'-dithiobis (2-nitrobenzoic acid), cysteine, trypsin inhibitor, phenylmethanesulfonyl fluoride, dimethyl sulfoxide, dihydroetide, lucigenin, nicotinamide adenine dinucleotide phosphate, and Griess reagent were obtained from Sigma-Aldrich (St. Louis, MO, USA). The other materials were of higher purity commercialized.

Vegetable material

Moringa oleifera seeds were collected in Recife, Pernambuco, northeastern Brazil, and stored at –20°C. A voucher (n° 73.345) was filed in the herbarium Dárdano de Andrade Lima at the Agronomic Institute of Pernambuco in Recife, Brazil. The plants were collected with authorization (n° 38690-2) from the Instituto Chico Mendes de Conservação da Biodiversidade from the Ministry of the Environment.

Water extract from the seeds of *Moringa oleifera*

The *M. oleifera* seeds were dried at 28°C and powdered using a blender. The powder (10 g) was homogenized with distilled water (100 mL) under constant agitation for 16 hours at 25°C. After filtration with cotton gauze and centrifugation (3,000 *g*, 15 min, 4°C), the supernatant corresponded to the WEMoS.

Isolation of WSMoL lectin

WSMoL was isolated as previously described by Coelho et al. (2009). Primarily, WEMoS was treated with ammonium sulfate at 60% saturation for 4 hours at 28°C, and the precipitated fraction was collected by centrifugation (3,000 *g*, 15 min, 4°C), resuspended and dialyzed against distilled water (4 h) and 0.15 M NaCl (4 h). The dialyzed fraction (50 mg of proteins) was then loaded onto a chitin column (7.5 × 1.5 cm) previously equilibrated (0.3 mL/min) with 0.15 M NaCl. Next, WSMoL was eluted with 1.0 M acetic acid and then dialyzed with distilled water (6 h, 4°C) to eliminate the eluent, yielding 3.4 mg per column. The protein concentration was determined according to Lowry et al. (1951) using bovine serum albumin as a standard. To check the presence of lectins around the process, hemagglutinating activity (HA) was evaluated as described by Procópio et al. (2017). Specific hemagglutinating activity (SHA) was calculated as the ratio between SHA and protein concentration.

Antioxidant activity by the ABTS test

The ABTS+ radical was generated by oxidizing the ABTS+ solution (7 mM) with 2.45 mM potassium persulfate solution. The mixture reacted for 12 hours in the dark at 25°C before use. For the test, the ABTS+ stock solution (1 ml) was diluted in 60 ml of methanol to obtain an absorbance of 0.70 ± 0.02 at 734 nm. Then, 2.7 mL of this diluted ABTS solution was added to a solution (0.3 mL) of WEMoS or WSMoL (both at 100, 125, 250, 500, and 1000 µg/mL) or to the standard Trolox (0.25, 0.5, 1.0, 2.0, and 4.0 µg/mL in methanol). The absorbance at 734 nm was read 6 minutes after adding the radical. The test was performed in triplicate to determine the percentage of inhibition of the ABTS radical and the concentration necessary to decrease the presence of ABTS+ by 50% (IC50).

Animals

The experimental design was approved by the Committee for Ethics in Animal Experimentation from the Universidade Federal de Pernambuco (authorization n° 29/15) and conducted according to Conselho Nacional de Controle de Experimentação Animal (CONCEA) guidelines, which comply with the ARRIVE guidelines and recognized international guidelines for the care and use of laboratory animals, as the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments. The animals were maintained in a 12-hour light/dark cycle, temperature 21°C ± 2°C, and with free access to water and feed.

Female Wistar rats aged 90 days were mated in a ratio of 3 females to 1 male. The pregnancy was confirmed by the presence of spermatozoids in the vaginal smear. The pregnant rats were placed in individual cages and randomly

separated into two groups. The Control group received subcutaneous administration of 0.9% NaCl (0.5 mL/kg body weight; C, n= 13) on days 13, 15, 17, and 19 of gestation, while the LPS dams received LPS (0.5 mg/kg body weight; L, n= 55). In addition, part of the LPS dams received the administration of WEMoS (200 mg/kg body weight; L+WEMoS, n= 13) or WSMoL (1.0 mg/kg body weight; L+WSMoL n= 15) from the gestation day 13 to 19. In addition, a group of LPS mothers was treated with a superoxide dismutase mimetic antioxidant, tempol (18 mg/kg body weight, drinking water; L+T, n= 12), and part of the LPS-dams received no treatment (L, n= 16).

Part of the dams of each group (C, n= 8; L, n= 11; L+WEMoS, n= 9; L+WSMoL, n= 9; and L+T, n= 6) was placed in metabolic cages on the 18th day of pregnancy, which was interrupted by surgical procedure on the 20^{th} day. The surgery occurred under thiopental anesthesia (60 mg/kg of body mass, via i.p.) to remove the maternal liver, male fetuses, and their placentas. As long as available, a maximum of two placentas and two male fetuses were used from each mother. The fetuses were weighed, and the livers and kidneys were removed.

Another part of the dams (C, n= 5; L, n= 5; L+WEMoS, n= 4; L+WSMoL, n= 5; and L+T, n= 6) had the pregnancy until term. Each litter was reduced to eight puppies, maintaining preferentially male rats, and following, they were weaned at 21 days of life. From the 90th day of life, the offspring were submitted to systolic blood pressure measurement every two weeks. At 120 days of age, the rats were euthanized under anesthesia (ketamine 80 mg/kg and xylazine 10 mg/kg, both via i.p.) to collect blood, kidney, and liver tissue. The kidney and liver were snap-frozen and stored at -80°C.

Plasma levels of urea, creatinine, and nitrite/nitrate

The plasma levels of urea and creatinine were measured in 120-day-old offspring using a commercial colorimetric test. The blood sample was collected by puncture of the aorta during euthanasia and maintained in tubes containing EDTA in an ice bath. The tubes were centrifuged at 10,000 *g* for 30 minutes plasma at 4°C.

The plasma levels of nitrite/nitrate were measured through the Griess reaction to estimate the plasma levels of NO. One volume of the Griess reagent (0.1% N-[1-naphthyl] ethylenediamine HCl, 1% sulfanilamide, and 5% H_3PO_4) was added to one volume of plasma and incubated for 10 minutes at room temperature. The absorbance was read at a wavelength of 540 nm, and the standard curve was built using $NaNO_2$.

Evaluation of systolic blood pressure

The offspring were submitted to assessment of systolic blood pressure (SBP) by tail-cuff plethysmography (IITC Life Science B60-7/16", Life Science Instruments) at 90, 110, and 120 days of age. For this, a pulse sensor coupled to a cuff was placed on the tail of awake rats. Following, the rats were heated to 32°C for 15 minutes, and then, five measurements of SBP were performed to obtain an average. Previously, the animals were adapted to the experimental procedures on three different days.

Investigation of the integrity of WSMoL in preparing the intestine of rats

The duodenum was homogenized for 15 minutes with 2 mL of 0.1 M Tris-HCl, pH 8.0, containing 20 mM $CaCl_2$, and then subjected to an ultrasonic bath for another 15 minutes. The suspension was centrifuged (9,000 *g*, 5 min, 4°C) to produce the homogenate. The protein concentration

was determined according to Lowry et al. (1951), and trypsin-like activity was assessed as described by De Oliveira et al. (2016) using the substrate BapNA. A unit of trypsin activity was defined as the amount of enzyme that hydrolyzes 1 µmol of BapNA per minute. The WSMoL (300 µg/1 mL) was incubated at 37 °C with the homogenate (1 mg protein/1 mL) for 1 and 2 hours at 37°C. After each incubation, protein digestion was interrupted by immersing the tubes in boiling water for 15 minutes. The mixtures were lyophilized and resuspended in 30 µL of the sample buffer and submitted to SDS-PAGE (12.5%, w/v, gel) according to Laemmli (1970). A control was also carried out containing the submitted lectin/homogenate mixture in the heating step without any incubation prior to 37°C. A sample containing only WSMoL was also electrophoresed on the same gel. The polypeptide bands were stained with Coomassie Brilliant Blue 0.02% (v/v) in 10% acetic acid (v/v).

Measurement of lipid peroxidation

Lipid peroxidation was assessed by measuring thiobarbituric acid reactive substances (TBARS) according to Ohkawa et al. (1979) in maternal liver and urine, placenta, fetal liver, and renal cortex and liver from adult offspring. The tissue was homogenized with 1.15% KCl and 3 mM EDTA in an ice bath. Subsequently, the homogenate (100 mg) was added to a reaction medium containing 0.3% thiobarbituric acid, 0.4% SDS, and 7.5% acetic acid (pH 3.5), and the mixture was heated to 95°C for one hour. The samples were centrifuged (1000 *g* for 10 min), and the absorbance of the supernatant was measured at a wavelength of 535 nm. The concentration of TBARS was calculated using 1,1,3,3-tetraethoxypropane as standard. The data were corrected by the protein concentration of the homogenate.

Basal production of superoxide anions and NADPH oxidase activity

The basal levels of superoxide anion production and the NADPH oxidase activity were evaluated by measuring lucigenin-derived chemiluminescence in the absence and presence of NADPH, respectively (Lima et al. 2021). First, the samples (placenta, fetal kidneys, and renal cortex of adult offspring) were homogenized in a buffer containing 150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS and 50 mM Tris, pH 8.0; containing 2 mM 4-(2-aminoethyl) benzene sulfonyl fluoride (AEBSF), 1 mM EDTA, 130 µM bestatin, 14 µM E-64, 1 µM leupeptin and 0.3 µM aprotinin. Next, the homogenates were added to a reaction medium containing 10 µM lucigenin and 100 µM NADPH. The luminescence was measured using a multimodular reader (Varioskan Flash, ThermoScientific, Waltham, USA) by performing 3-sec measurement cycles every 30 seconds for 5 minutes. The result was expressed in relative light units per minute and corrected by the amount of protein in the sample.

Reactive oxygen species levels

The reactive oxygen species (ROS) levels were measured using the fluorescent probe dihydroethidium (DHE) in samples from the renal cortex of the adult offspring. First, the renal cortex was homogenized in a buffer containing 150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, and 50 mM Tris, pH 8.0; containing 2 mM AEBSF, 1 mM EDTA, 130 µM bestatin, 14 µM E-64, 1 µM leupeptin and 0.3 µM aprotinin. Following, the homogenate was incubated with PBS, 80 mM diethylenetriaminepentaacetic acid (DTPA), and 5 µM DHE for 20 minutes at 37°C, protected from light. The fluorescence was measured at an excitation/emission wavelength of 500/580 nm using a multimodular reader (Varioskan Flash,

ThermoScientific). The result was corrected by the protein concentration of the sample.

Statistical analysis

The results are expressed by the mean ± standard error of the mean (SEM). The data were submitted to confirmation of normality distribution using the Shapiro-Wilk test. Considering the absence of significant variations in the Gaussian distribution, the means of the groups were compared using one-way analysis of variance (ANOVA) followed by the Tukey test. The analyses were performed using the GraphPad Prism 5 software (version 5.01, GraphPad Software, Inc.). Differences between groups were considered significant at $P<0.05$.

RESULTS

Purification of WSMoL

WEMoS showed a protein concentration of 8.35 mg/mL and a SHA of 1.91. Isolated WSMoL presented a SHA of 1,462, revealing a purification factor of 765.

In vitro antioxidant effects of WEMoS and WSMoL

The ability of WEMoS and WSMoL to react with ABTS+ was compared with that of Trolox. The results are shown in Table I. While the IC50 of Trolox was 1.76 ± 0.08 µg/mL, the IC50 for WEMoS and WSMoL were 750.2 ± 6.58 and 742.6 ± 17.6 µg/mL, respectively.

Effect of rat duodenum enzymes on WSMoL

The duodenum homogenate showed trypsin-like activity (11.3 ± 3.2 mU/mg), confirming that active proteases were present during the assays. Figure 1 shows the SDS-PAGE of mixtures containing WSMoL and duodenum homogenates without incubation (lane 1) or after incubation at 37°C for 1 or 2 hours (lanes 2 and 3, respectively). A sample containing only WSMoL was also electrophoresed on the same gel (lane 4), revealing two polypeptide bands consistent with the SDS-PAGE profile previously reported for this lectin (De Moura et al. 2016, De Oliveira et al. 2016). In addition, WSMoL polypeptide bands were detected in the samples incubated for 1 and 2 hours; however, the intensity was lower than that observed in the unincubated mixture. This result demonstrates that this lectin exhibits good resistance to digestion by duodenum proteases.

Maternal and fetal functional parameters

The group submitted to LPS administration presented lower (27%, P<0,001) maternal weight gain and nearly 15% lower ($P<0.05$) placental and fetal kidney weight (Table II). The fetal body weight was not altered by LPS administration, albeit the maternal food intake was more than 20% lower ($P<0.01$). The LPS dams treated with WEMoS or WSMoL did not present a reduction of body weight gain and placental weight compared to the Control group. Conversely, the

Table I. Antioxidant effects of WEMoS and WSMoL on ABTS+.

Trolox			WEMoS	WSMoL
Concentration (µg/mL)	**ABTS+ inhibition (%)**	**Concentration (µg/mL)**	**ABTS+ inhibition (%)**	**ABTS+ inhibition (%)**
4.0	92.06 ± 0.27	**1000**	57.51 ± 0.70	60.16 ± 2.05*
2.0	60.07 ± 0.24	**500**	46.12 ± 0.23	45.42 ± 2.89
1.0	35.82 ± 0.27	**250**	33.25 ± 0.07	30.02 ± 0.23*
0.5	24.10 ± 0.08	**125**	16.27 ± 0.62	15.14 ± 0.54
0.25	16.69 ± 0.41	**100**	18.7 ± 0.08	9.86 ± 0.14*

***P<0.05 WSMoL vs. WEMoS.**

treatment did not prevent the reduction of food intake induced by LPS.

WEMoS and WSMoL prevented the elevation of maternal oxidative stress induced by LPS administration

In Figure 2a, b, it is shown the levels of lipid peroxidation in the urine and liver from dams. Maternal endotoxemia increased the levels of MDA by 34% (P<0.01) and 86% (P<0.01) in the urine and liver, respectively, in comparison to the control dams. The LPS groups treated with WEMoS, WSMoL, or tempol presented lower urine lipid peroxidation than untreated LPS rats: 50% (P<0.001), 40% (P<0.001), and 25% (P<0.01), respectively. Moreover, the lipid peroxidation in the liver of WEMoS and WSMoL-treated rats was not different from either Control or untreated LPS rats. Conversely, the hepatic MDA levels of tempol-treated rats were 45% lower (P<0.01) than the LPS group.

The elevation of superoxide anion levels and NADPH oxidase activity in the placenta programmed by maternal endotoxemia were reduced by WEMoS and WSMoL

Figure 3a, b, and c shows the placenta's lipid peroxidation, basal superoxide anions production, and NADPH oxidase activity. The maternal endotoxemia did not increase the MDA levels of the placenta. However, the placenta from LPS groups treated with WEMoS, WSMoL, and tempol presented lower MDA levels than LPS rats without treatment (Figure 3a). Additionally, the lipid peroxidation of placentas from the LPS group treated with WSMoL was more than 50% lower (P<0.01) than placentas from the WEMoS group.

The basal superoxide anions production of the placenta increased more than 1 time (P<0.001) due to LPS administration (Figure 3b). The LPS groups that received WEMoS, WSMoL, or tempol presented lower levels of placenta

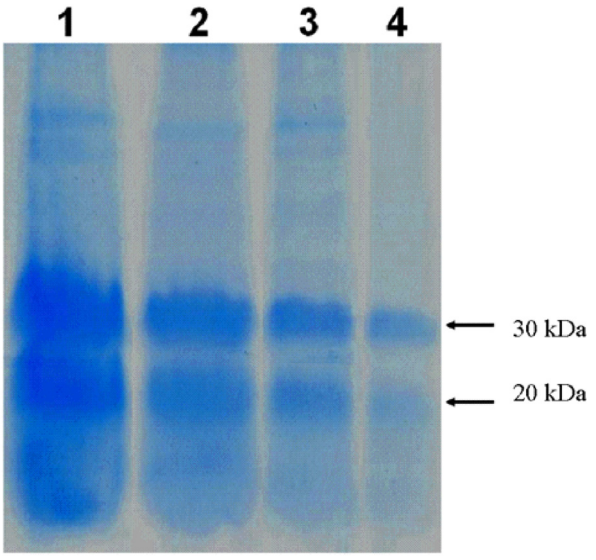

Figure 1. Representative images of WSMoL in SDS-PAGE. Lanes 1, 2, and 3 represent lectin incubated with rat duodenum homogenate at 37°C for 0, 1, and 2 h, respectively. Lane 4 contains only lectin. The arrows indicate the polypeptide bands corresponding to the SDS - PAGE profile of WSMoL.

superoxide anions production compared to the untreated LPS group. Moreover, the superoxide anions production in the placenta of tempol and WSMoL-treated dams was lower than in the WEMoS-treated group. In the same way, as in superoxide anions production, the LPS administration increased more than 1 time (P<0.001) the NADPH oxidase activity in the placenta (Figure 3c). The WEMoS treatment did not reduce the stimulation of NADPH oxidase activity induced by LPS. Conversely, WSMoL and tempol-treated dams presented more than 50% lower (P<0.001) NADPH oxidase activity than the untreated LPS group.

The antioxidant effects of WEMoS and WSMoL in the fetus from LPS-treated dams

In the liver of the fetuses at 20 days of gestation, the lipid peroxidation was 80% higher (P<0.001) in the LPS group compared to Control (Figure 4a). The treatment with WEMoS and WSMoL reduced by 25% (P<0.001) and 75% (P<0.001), respectively, the levels of MDA in LPS fetuses, being the values

Table II. Maternal and fetal parameters.

	C	L	L+WEMoS	L+WSMoL	L+T
Maternal weight gain (g)	94 ± 5	69 ± 2*	79 ± 2*†	74 ± 4*	55 ± 1*†‡§
Placental weight (g)	0.53 ± 0.01	0.46±0.01*	0.50 ± 0.01†	0.48 ± 0.01*	0.53 ± 0.01†§
Fetal body weight (g)	3.77 ± 0.02	3.74 ± 0.05	3.77 ± 0.04	3.47 ± 0.07	3.12 ± 0.24*†‡
Fetal liver weight (g)	0.28 ± 0.01	0.24 ± 0.01*	0.25 ± 0.01	0.23 ± 0.01*	0.24 ± 0.01
Fetal kidney weight (mg)	14 ± 1	12 ± 1*	15 ± 1†	12 ± 1*‡	12 ± 1*‡
Maternal food intake (g/day)	28 ± 1	22 ± 1*	20 ± 1*	21 ± 1*	21 ± 1*
Maternal water intake (mL/day)	59 ± 5	51 ± 3	55 ± 5	44 ± 3	52 ± 2

Data are presented as mean ± SEM. The gain in maternal body mass represents the difference in body weight between gestation day 20 and 0. C: control group; L: dams that received LPS during pregnancy; L+WEMoS: LPS dams treated with WEMoS (200 mg/kg body weight); L+WSMoL: LPS dams treated with WSMoL (1.1 mg/kg body weight); and L+T: LPS dams treated with tempol (18 mg/kg body weight). The comparison between the means of the groups was performed by the one-way analysis of variance followed by the Tukey test: *P<0.05 vs. Control (C); †P<0.05 vs. LPS (L); ‡P<0.05 vs. L+WEMoS; §P<0.05 vs. L+WSMoL.

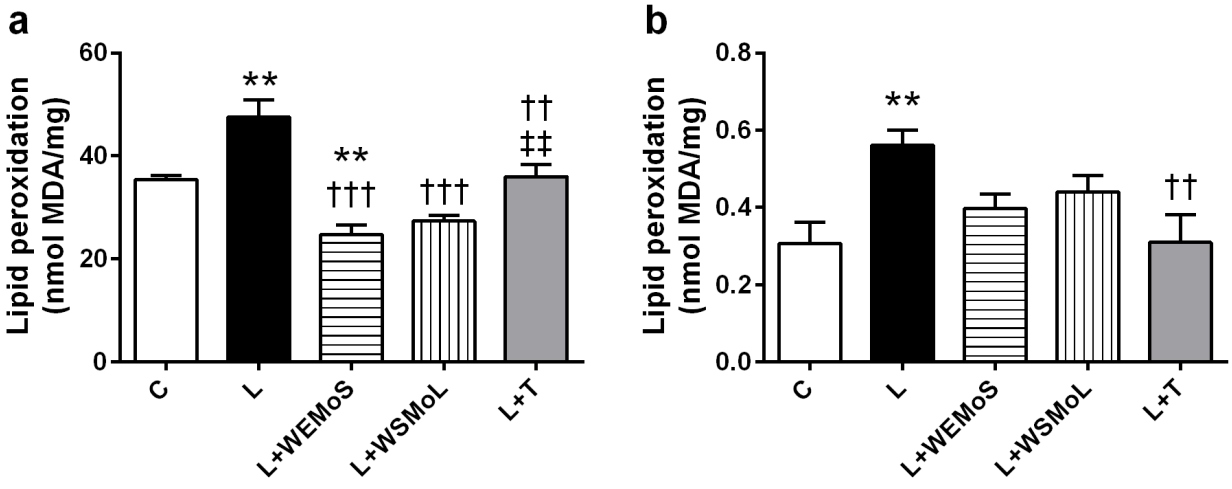

Figure 2. Effects of the treatment with water-extract of *Moringa oleifera* seeds (WEMoS) and with water-soluble *Moringa oleifera* lectin (WSMoL) on the levels of lipid peroxidation in the urine (a) and liver (b) of pregnant rats submitted to endotoxemia at gestation day 20. C: control group; L: dams that received LPS during pregnancy; L+WEMoS: LPS dams treated with WEMoS (200 mg/kg body weight); L+WSMoL: LPS dams treated with WSMoL (1.1 mg/kg body weight); and L+T: LPS dams treated with tempol (18 mg/kg body weight). Data are expressed as mean ± SEM. The experimental *n* ranged between 5-11. The comparison between the means of the groups was performed by the one-way analysis of variance followed by the Tukey test: **P<0.01 Vs. Control (C); ††P<0.01 and †††P<0.001 vs. LPS (L); ‡‡P<0.01 vs. L+WEMoS.

of WSMoL group 60% lower (P<0.001) than the values of WEMoS fetuses.

In the same way, as occurred in liver lipid peroxidation, the administration of LPS increased the basal level of superoxide anion production and the activity of NADPH oxidase of the fetal kidney by more than 200% (P<0.001) (Figure 4b and 4c). The superoxide anion production of the fetal kidney of the WEMoS group was not different from Control fetuses, while the NADPH oxidase activity was 170% higher (P<0.001). On the other hand, the WSMoL treatment did not change the level of fetal kidney superoxide anions compared to non-treated LPS fetuses. Still, it decreased by 70% (P<0.001) the NADPH oxidase activity. The administration of tempol

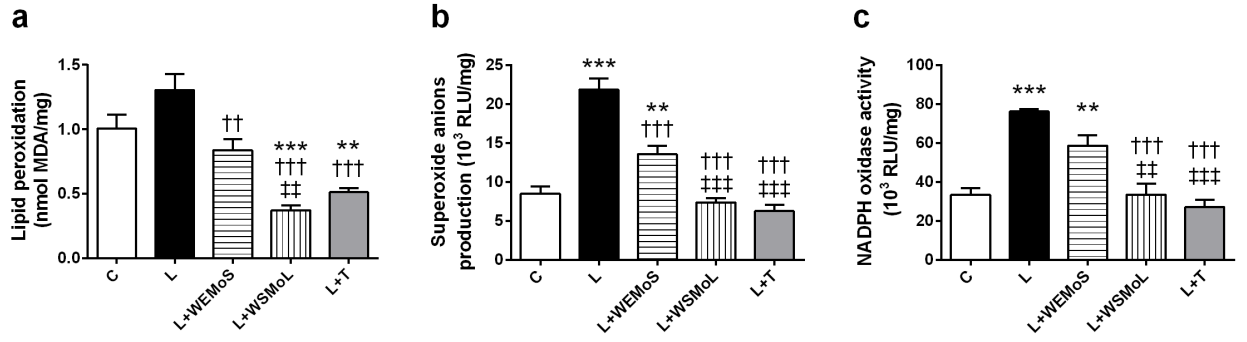

Figure 3. Effects of the treatment with water-extract of *Moringa oleifera* seeds (WEMoS) and with water-soluble *Moringa oleifera* lectin (WSMoL) on the lipid peroxidation (a), superoxide anions production (b), and NADPH oxidase activity (c) in the placenta from rats submitted to endotoxemia during gestation. The groups of dams are the same, as depicted in the legend of Figure 2. Data are expressed as mean ± SEM. The experimental *n* ranged between 6-9. The comparison between the means of the groups was performed by the one-way analysis of variance followed by the Tukey test: **P<0.01 and ***P<0.001 Vs. Control (C); ††P<0.01 and †††P<0.001 vs. LPS (L); ‡‡P<0.01 and ‡‡‡P<0.001 Vs. L+WEMoS.

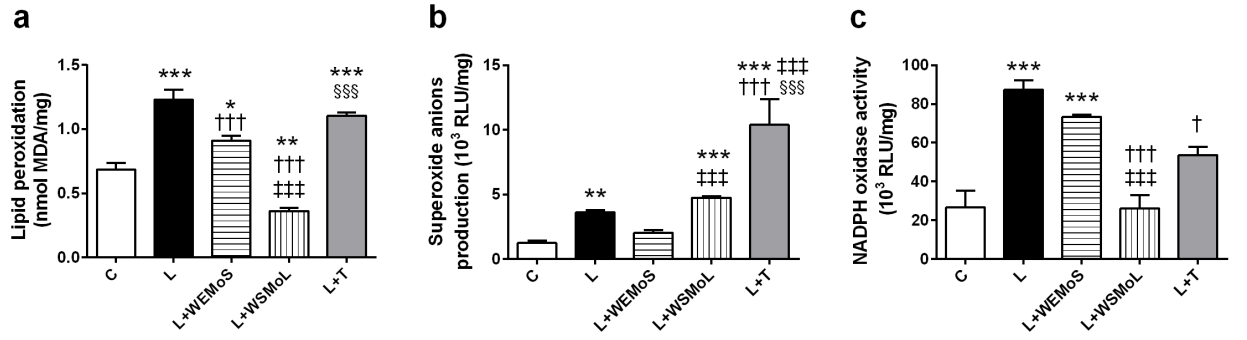

Figure 4. Effects of the treatment with water-extract of *Moringa oleifera* seeds (WEMoS) and with water-soluble *Moringa oleifera* lectin (WSMoL) on fetuses obtained from mothers submitted to endotoxemia: liver lipid peroxidation (a); renal basal superoxide anions production (b); and renal NADPH oxidase activity (c). C: fetuses from the control group; L: fetuses from dams that received LPS during pregnancy; L+WEMoS: fetuses from LPS dams treated with WEMoS (200 mg/kg body weight); L+WSMoL: fetuses from LPS dams treated with WSMoL (1.1 mg/kg body weight); and L+T: fetuses from LPS dams treated with tempol (18 mg/kg body weight). Data are expressed as mean ± SEM. The experimental *n* ranged between 3-11. The comparison between the means of the groups was performed by the one-way analysis of variance followed by the Tukey test: *P<0.05, **P<0.01, and ***P<0.001 Vs. Control (C); †P<0.05 and †††P<0.001 vs. LPS (L); ‡‡‡P<0.001 Vs. L+WEMoS; §§§P<0.001 Vs. L+WSMoL.

increased the hepatic levels of MDA and the renal superoxide anion compared to Control fetuses. However, it did not increase NADPH oxidase activity.

WEMoS and WSMoL prevented LPS-induced hypertension and elevation of serum creatinine

As seen in Figure 5a, the LPS-exposed offspring presented higher SBP than the Control rats throughout the evaluation period. For example, in 120-day-old rats, the SBP was 25% (P<0.001) higher than the control group (Figure 5b). In addition, the offspring from LPS dams treated with WEMoS presented lower SBP than LPS non-treated rats at ages 100 and 120 days; however, these values remained higher than the Control group. Conversely, the offspring from LPS dams treated with WSMoL or Tempol presented lower

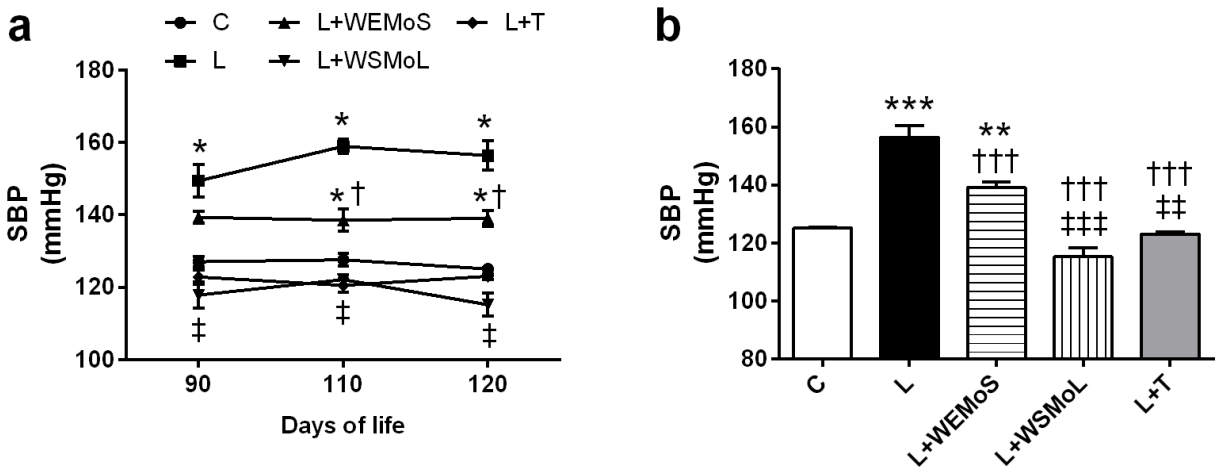

Figure 5. Effects of the treatment with water-extract of *Moringa oleifera* seeds (WEMoS) and with water-soluble *Moringa oleifera* lectin (WSMoL) on the systolic blood pressure of adult offspring obtained from mothers submitted to endotoxemia during gestation: time-course (a) and end-point (b) SBP evaluated between 90 and 120 days of age. C: offspring from the Control group; L: offspring from dams that received LPS during pregnancy; L+WEMoS: offspring from LPS dams treated with WEMoS (200 mg/kg body weight); L+WSMoL: offspring from LPS dams treated with WSMoL (1.1 mg/kg body weight); and L+T: offspring from LPS dams treated with tempol (18 mg/kg body weight). Data are expressed as mean ± SEM. The experimental n ranged between 4-6. The comparison between the means of the groups was performed by the one-way analysis of variance followed by the Tukey test: *P<0.05, **P<0.01, and ***P<0.001 Vs. Control (C); †P<0.01 and †††P<0.001 vs. LPS (L); ‡‡P<0.01 and ‡‡‡P<0.001 Vs. L+WEMoS.

SBP levels than the non-treated LPS group in all periods studied (Figure 5a and 5b).

It was also evaluated the effects of WEMoS and WSMoL on the levels of serum markers of renal function: urea and creatinine. The serum urea levels were not different between experimental groups (data not shown). On the other side, the groups treated with WEMoS and WSMoL presented lower serum creatinine concentration (P<0.001) than the LPS group without treatment (WEMoS: 0.47 ± 0.03; WSMoL: 0.32 ± 0. 08; and L: 0.80 ± 0.03 mg/dL).

Long-term antioxidant effects of WEMoS and WSMoL in the kidney and liver of adult offspring

The LPS administration increased the renal cortex and the liver levels of malondialdehyde by nearly 40% (P<0.01) in comparison to Control rats (Figure 6a and 6b). The treatment with WSMoL and WEMoS prevented the elevation of renal lipid peroxidation induced by LPS. On the other hand, the WSMoL treatment completely prevented the increase of lipid peroxidation in the liver, while the protective effect of WEMoS was partial; the MDA levels were lower than LPS and higher than the control groups. Tempol treatment did not prevent the LPS-induced changes in liver lipid peroxidation.

The maternal endotoxemia increased by 70% (P<0.001) of the renal ROS production in the adult offspring (Figure 6c). However, all the treatments prevented the increase of renal ROS induced by LPS. The same profile of response to LPS and treatments was observed in the basal superoxide anion production and NADPH oxidase activity evaluated in the renal cortex of adult offspring (Figure 6d and 6e).

The plasma levels of nitrate/nitrite were 44% higher (P<0.05) in the LPS group compared to the Control (Figure 7). Conversely, the groups treated with WEMoS, WSMoL, and Tempol

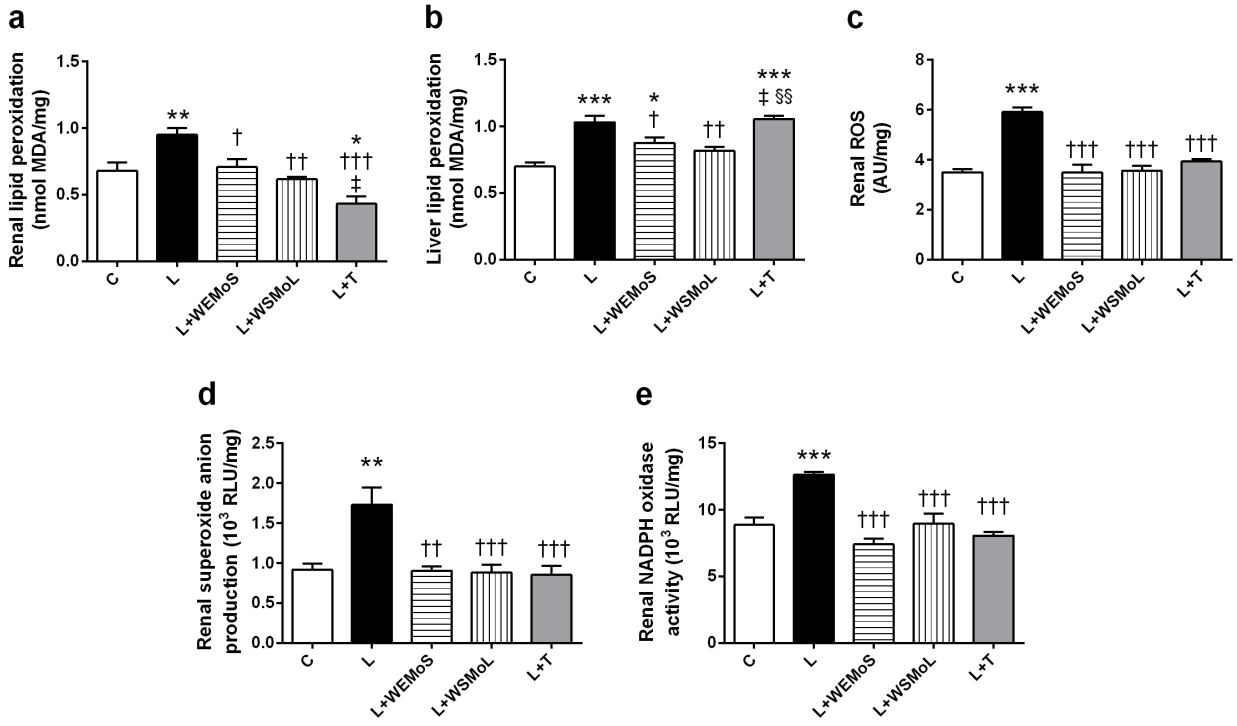

Figure 6. Effects of the treatment with water-extract of *Moringa oleifera* seeds (WEMoS) and with water-soluble *Moringa oleifera* lectin (WSMoL) on renal and hepatic oxidative stress of adult rats: renal lipid peroxidation (a), liver lipid peroxidation (b), renal levels of reactive oxygen species (ROS) (c), renal superoxide anions production (d), and renal NADPH oxidase activity (e). The groups of rats are the same as depicted in the legend of Figure 5. Data are expressed as mean ± SEM. The experimental *n* ranged between 4-9. The comparison between the means of the groups was performed by the one-way analysis of variance followed by the Tukey test: *P<0.05, **P<0.01, and ***P<0.001 Vs. Control (C); and †P<0.05, ††P<0.01, and †††P<0.001 vs. LPS (L); ‡P<0.05 vs. LPS+WEMoS; §§P<0.01 Vs. L+WSMoL.

presented lower plasma levels of nitrate/nitrite compared to the untreated LPS group.

DISCUSSION

Maternal endotoxemia is a condition related to the elevation of oxidative stress in the fetal-maternal environment that affects renal development and induces hypertension in the adult offspring. Early antioxidant treatment can prevent the intrauterine programming of hypertension; however, under control conditions, some antioxidant substances also influence the phenotype of the fetus and increase the risk of late adverse events (Vieira-Filho et al. 2014, Ribeiro et al. 2018). Thus, it is crucial to identify substances with antioxidant action in vivo that do not impair the mother or fetus.

WSMoL was isolated with a high purification factor regarding the water extract, as already reported in previous studies. This datum indicates that the WSMoS contains several other proteins than WSMoL. Previous study showed that the water extract of *Moringa oleifera* seeds contains, in addition of lectins, secondary metabolites belonging to the classes of alkaloids, flavonoids, phenylpropanoids, saponins, and tannins, as well as reducing sugars. In addition, HPLC analysis confirmed the presence of ellagic acid and rutin (Oliveira et al. 2020).

The present study demonstrates that the WEMoS and WSMoL presented *in vivo* antioxidant

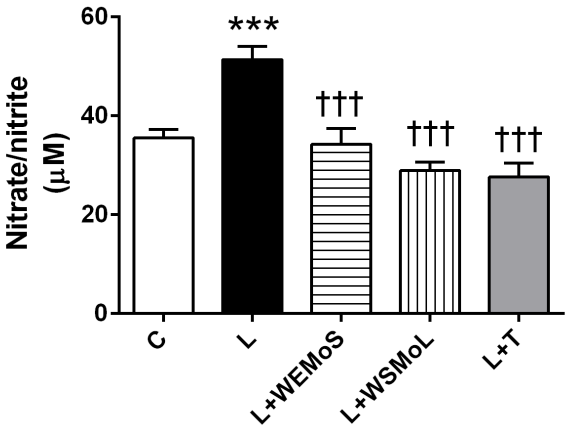

Figure 7. Effects of the treatment with water-extract of *Moringa oleifera* seeds (WEMoS) and water-soluble *Moringa oleifera* lectin (WSMoL) on the plasma levels of nitrate/nitrite in adult rats from mothers submitted to endotoxemia. The groups of rats are the same as depicted in the legend of Figure 5. Data are expressed as mean ± SEM. The experimental *n* ranged between 3-6. The comparison between the means of the groups was performed by the one-way analysis of variance followed by the Tukey test: ***P<0.001 Vs. Control (C); and †††P<0.001 vs. LPS (L).

effects. The WEMoS and the WSMoL lowered the lipid peroxidation in the maternal and fetal environment, and this effect reverberated in the adult life of the offspring by preventing hypertension. The antioxidant effects of WEMoS or WSMoL may be partially attributed to NADPH oxidase inhibition.

The maternal treatment with WEMoS prevented the reduction of maternal body weight gain induced by LPS. Moreover, the treatment prevented decreased placental and fetal kidney weights. The protective effects of WEMoS on maternal body weight gain and placenta growth may be essential to improve intrauterine development and occur independently to increased maternal food intake. Therefore, the recovery of maternal weight gain may rely on the extract composition, which is rich in proteins, essential amino acids, minerals, vitamins, and other bioactive compounds (Moyo et al. 2012, Ribeiro et al. 2018).

The effects of WEMoS and WSMoL in preventing LPS-induced oxidative stress were well characterized in pregnant rats, similarly to the effects induced by the superoxide dismutase mimetic tempol. Considering that WEMoS prevented the elevation of oxidative stress in the liver of dams and placenta, it is likely that the protective effects of the extract on placenta growth also include antioxidant actions. It is known that antioxidant treatment may improve placenta blood flow and, therefore, fetal development (Valdez-Solana et al. 2015). In addition, *M. oleifera* can prevent or decrease the speed of oxidation of other molecules, usually by scavenging free radicals and reducing the development of inflammatory cytokines due to their high phenolic content (Costa et al. 2016, Alhakmani et al. 2013, Kumar et al. 2014, Singh et al. 2009). Moreover, the antioxidant action of WEMoS has already been reported in rats (Singh et al. 2013), although with a higher dose than the presently used (1 g/kg).

Identifying the in vitro antioxidant activity of WSMoL points to its importance as a direct ROS scavenger and may be partially responsible for the in vivo antioxidant action of WEMoS. It has already been demonstrated that WSMoL presents in vivo anti-inflammatory effects (Araújo et al. 2013); however, this is the first report to show the antioxidant action of WSMoL in vivo. The dose of WSMoL was chosen based on its estimated presence in WEMoS, considering its dose of 200 mg/kg. Treatment with WSMoL did not recover maternal weight gain and fetal renal weight; however, it presented antioxidant effects in the mother (liver and urine), placenta, and fetus (liver). This observation indicates that WSMoL can also positively impact placental perfusion and fetal growth (Valdez-Solana et al. 2015).

Although both WEMoS and WSMoL prevented oxidative stress and decreased ROS in the

maternal and fetal environment, only WSMoL inhibits placental and renal NADPH oxidase activity. This is another factor that illustrates the different mechanisms of action between the two preparations. Therefore, the antioxidative effect of WSMoL may be dependent on NADPH oxidase inhibition, a valuable feature of antioxidant therapy, because unspecific antioxidant substances may cause adverse responses in some situations due to ROS-dependent physiologic signaling (Holmström & Finkel 2014, Vieira-Filho et al. 2014, Ribeiro et al. 2018).

In addition to oxidative stress being critical to intrauterine growth retardation, it is also crucial in the pathogenesis of hypertension in adult life (Vieira et al. 2018, Griendling et al. 2021). Although there were divergences in the effects of treatments on some maternal parameters, the three treatments (WEMoS, WSMoL, and tempol) prevented the programming of hypertension induced by the administration of LPS. This demonstrates the importance of oxidative stress in the intrauterine programming mechanism and illustrates that the protective antioxidant action of WEMoS and WSMoL is comparable to that of the superoxide dismutase mimetic.

The ROS present a wide range of mechanisms that may increase arterial pressure by modulation of cardiovascular and renal systems (Griendling et al. 2021). Here, it was observed that the maternal treatment with WEMoS and WSMoL decreased the renal ROS production and the renal oxidative stress in the adult offspring (Figure 6) in the same way as it prevented the elevation of the blood pressure (Figure 5). It is important to emphasize that the protective effects of the treatments were long-term, considering that the treatment was restricted to the period of fetal development.

The long-term antioxidative effects of maternal WEMoS and WSMoL administration may partially depend on the modulation of renal NADPH oxidase. Both treatments prevented the elevation of renal NADPH oxidase in the adult offspring. This effect mirrored what was observed in the maternal-fetal environment, suggesting that the prevention of increased renal NADPH oxidase activity was maintained during offspring growth. The modulation of NADPH oxidase may also be essential to preventing hypertension programming by maternal LPS. This can be evidenced in two ways: i) adult rats from mothers submitted to LPS administration present higher renal NADPH oxidase content and activity (Vieira et al. 2018, Farias et al. 2020), and ii) the administration of the NADPH oxidase inhibitor apocynin blunts the elevation of blood pressure programmed by maternal LPS (Vieira et al. 2018).

The protection against hypertension development in the offspring obtained from LPS-treated dams may also be due to anti-inflammatory effects. It is known that maternal LPS exposure increases the production of inflammatory cytokines, which are delivered to a greater extent to the placenta and fetus (Tak & Firestein 2001). WSMoL treatment may prevent this change because the lectin can lower TNF-α and NO levels in LPS-stimulated macrophages (Araújo et al. 2013). Maternal LPS exposure also resulted in positive regulation of plasma nitrate/nitrite levels, an indicator of higher NO production and inflammation, in the adult rat (Saijo et al. 2010). This indicates that pro-inflammatory pathways were activated to potentiate the effects of oxidative stress in the offspring in adult life. On the other hand, it was also shown that treatment with WSMoL and WEMoS can protect against the late change in NO levels, even considering that the treatment was administered only to mothers. This protective effect was similar to that observed in the redox balance modulation.

The present study demonstrates that WEMoS and WSMoL have protective effects on maternal endotoxemia, which involve antioxidant and anti-inflammatory actions that prevent the programming of hypertension. Moreover, the data indicate that the antioxidative effects of WEMoS are partially dependent on WSMoL content and occur through the inhibition of NADPH oxidase. Thus, the possibility of using both preparations in clinical situations involving activating pro-inflammatory and pro-fibrotic pathways is feasible. However, it is still important to better understand the differences in the mechanism of action between the two preparations.

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