Journal of Ethnopharmacology 261 (2020) 113150

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

The ethanolic extract of *Terminalia argentea* Mart. & Zucc. *bark* reduces the inflammation through the modulation of cytokines and nitric oxide mediated by the downregulation of NF-κB

Mirella dos Reis de Araújo Moreira[a,1], Helioswilton Sales-Campos[b,c,1], Caroline Fontanari[a], Alyne Fávero Galvão Meireles[a], Morgana Kelly Borges Prado[a], Karina Furlani Zoccal[a,d], Carlos Artério Sorgi[a], Cristiane Tefé da Silva[d], Milton Groppo[e], Lúcia Helena Faccioli[a,*]

[a] *Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil*
[b] *Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil*
[c] *Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, Goiás, Brazil*
[d] *Centro Universitário Barão de Mauá, Ribeirão Preto, São Paulo, Brazil*
[e] *Departamento de Biologia, Faculdade de Filosofia Ciências e Letras de Ribeirão Preto, Ribeirão Preto, São Paulo, Brazil*

ARTICLE INFO

Keywords:
Terminalia argentea
Inflammation
Anti-inflammatory activity
Cytokines
NF-κB
Air-pouch

ABSTRACT

Ethnopharmacological relevance: *Terminalia argentea* Mart. & Zucc. (Combretaceae), popularly known as "capitão do campo", is native from the Brazilian cerrado, which is used in folk medicine to treat inflammatory diseases.
Aim of the study: We aimed to investigate the anti-inflammatory effects, toxicity and mechanisms of action regarding the use of the hydroalcoholic extract of *T. argentea* bark.
Materials and methods: Toxicity was determinate *in vitro* using the macrophage lineage J774.1 without LPS. Cells were treated with 0.5; 2; 8; 32 and 125 μg/mL of the plant extract. Cell viability was assessed by MTT colorimetric assay. The production of nitrite and cytokines was also determined in the supernatants. A NF-κB reporter assay using RAW macrophages was employed to elucidate the impact of the plant extract on the expression of such molecule. In mice, toxicity was assessed by orally given an intermediate to high concentration of the plant extract on a single dose (1000 or 5000 mg/kg) or low and intermediate doses (300 or 1000 mg/kg) twice daily for 14 days. Blood samples were collected for biochemical analysis. The anti-inflammatory activity was assessed using the air-pouch model with or without pre-inoculation with the inflammatory stimuli LPS (0.5 μg/mL), followed by treatment with plant extract at 5, 60 or 300 mg/kg administered in the air pouch (subcutaneous injection). After 4 h, mice were euthanized and the air pouches washed with 2 mL heparinized PBS (10 IU/mL). Then, the local production in the air pouch wash of cytokines, total proteins and leukocytes was assessed.
Results: No signals of toxicity were observed either in cells or mice. Regardless the concentration used *in vitro,* the extract exhibited a significant anti-inflammatory activity, as perceived by the reduction of the inflammatory cytokines IL-1β, TNF-α and IL-6 and nitrites on cell supernatants. This was concomitant with a downregulation in NF-κB and elevated levels of IL-10. In mice, similar effects were observed, especially when the plant extract was given at 300 mg/kg, inhibiting the release of IL-1β, TNF-α, IL-6 and proteins, as well as increasing the release of IL-10.
Conclusions: Altogether, our results demonstrated that the hydroalcoholic extract of *T. argentea* bark has anti-inflammatory activity without inducing toxicity in cells or living animals. This activity seems to be chiefly influenced by a downregulation in NF-κB, inflammatory cytokines and production of nitrite along with augmented concentration of IL-10.

* Corresponding author. Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo (USP), Av. do Café, s/n, 14040-903, Ribeirão Preto, SP, Brazil.

E-mail addresses: mirekka@yahoo.com.br (M.R. de Araújo Moreira), tonsales@ufg.br (H. Sales-Campos), fontanari@usp.br (C. Fontanari), alynefg@fcfrp.usp.br (A.F. Galvão Meireles), morgana.kelly@hotmail.com (M.K. Borges Prado), karina_zoccal4@hotmail.com (K.F. Zoccal), sorgi@fcfrp.usp.br (C.A. Sorgi), cris_tefe@hotmail.com (C. Tefé da Silva), groppo@ffclrp.usp.br (M. Groppo), faccioli@fcfrp.usp.br (L.H. Faccioli).

[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.jep.2020.113150
Received 7 May 2020; Received in revised form 18 June 2020; Accepted 2 July 2020
Available online 27 July 2020

0378-8741/© 2020 Elsevier B.V. This article is made available under the Elsevier license (http://www.elsevier.com/open-access/userlicense/1.0/).

1. Introduction

Popular use of plants in traditional medicine represents a great source of discovering molecules with therapeutic effect. In this context, natural molecules isolated from medicinal plants have long been used to treat different inflammatory conditions. Brazil has its territory occupied by 6 terrestrial biomes in which about one fourth of the world's known species can be found with great abundance and diversity. Among them, the family Combretaceae, native from one of the biomes, the Brazilian cerrado, is widely used in folk medicine to treat inflammatory conditions (Ribeiro et al., 2018).

Experimental studies conducted with different members from family Combretaceae, such as *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn. (Halder et al., 2009), *T. paniculata* Roth (Talwar et al., 2011). and *T. catappa* L. (Yeh et al., 2012), have presented promising results regarding the anti-inflammatory effect of such extracts. Another important member of this family, *T. argentea* Mart. & Zucc., also known as "capitão do campo", "capitão do cerrado" or "capitão", have been effectively used in popular medicine to treat inflammatory disturbances in respiratory (e.g. pneumonia and bronchitis) and urinary (cystitis) tracts (Fahmy et al., 2015). In riverine communities located in the Midwest region of Brazil, *T. argentea* has been used to treat several illnesses. As infusion, leaves have been effectively used to treat gastric ulcers, bronchitis and hemorrhages. The bark, in different preparations, has been employed by these communities to treat inflammatory and infection-related conditions such as ulcers, fever, diarrhea and cancer (Ribeiro et al., 2017). The ethanolic extract of *T. argentea* bark has demonstrated the occurrence of pentacyclic triterpenes, flavones and lignans (Fahmy et al., 2015). In general, these compounds are frequently found in plants, fruits and vegetables besides of presenting anti-inflammatory, anti-infectious and anti-tumoral effects (Allouche et al., 2009; During et al., 2012; Singh et al., 2014).

Despite the beneficial effects of the ethanolic extract of *T. argentea* bark, its toxicity and especially the mechanism of action, have never been addressed before. Thus, the aim of this study was to evaluate cytotoxicity and the anti-inflammatory mechanisms related to the compounds in the ethanolic extract of *T. argentea* bark both using *in vitro* and *in vivo* approaches.

2. Methods and materials

2.1. Plant material and preparation of the extract

Samples of *T. argentea* Mart. & Zucc. bark were collected in the city of Uberaba, state of Minas Gerais, Brazil (Lat. −19.743623, Long −47.828514). All samples were analyzed and identified by Prof. Dr. Milton Groppo and deposited in the herbarium of the Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras of Ribeirão Preto, Universidade de São Paulo (Herbarium SPFR) under the collector number Groppo 2468 and herbarium record number 17773. The extracts were obtained from 100 g of ground and dry bark, dissolved in 1000 mL of 70% ethanol (JT Baker, Belo Horizonte, Brazil). A dark bottle conditioned at room temperature was used for 72 h with daily shaking, and the extract was then filtered on filter paper. The filtrate was dried using a rotary evaporator (Eppendorf Vacuum Concentrator Plus, Hamburg, Germany). The dried extract was weighed and suspended in 1.000 mL of PBS (phosphate-buffered saline) containing 0.3% (v/v %) DMSO (Dimethyl sulfoxide, Sigma-Aldrich, Missouri, USA) until the concentration of 1 g/mL. This extract was filtered and stored in a freezer at −20 °C in 500 μL aliquots until used.

2.2. Cell culture and in vitro experimental design

The J774.1 murine macrophage lineage was purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). The cells were plated in 96-well culture plates (Corning Inc., Corning, NY, USA) at 1×10^5 cells/well in DMEM medium supplemented with 10% fetal bovine serum (DMEM-c) and 1% gentamicin in an incubator with a moist atmosphere of 5% CO_2 at 37 °C. After the formation of a monolayer, the plate was gently centrifuged at 150 *g* (Beckman, Indianapolis, IN, USA) for 10 min at 10 °C. Then, the supernatant was gently removed and replaced by 200 μL of DMEM-c containing different concentrations of the plant extract (31.25, 62.5, 125, 250, 500 and 1000 μg/mL). After 24 h of incubation, cell viability was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay (Sigma-Aldrich, Hamburg, Germany). Next, the cultures were incubated with 5% MTT in DMEN-c for 3 h. Subsequently, 50 μL of sodium dodecyl sulfate 20% in 0.01 M HCl were added to each well and maintained at room temperature until complete precipitate solubilization. Absorbance was measured at 570 nm with a spectrophotometer (mQuanti, Bio-Tek Instruments, Inc., Winooski, VT) and was positive correlated with cell viability. As a positive control DMSO was used, which kills 100% of the cells when in concentrations higher than 3 μg/mL.

For determination of anti-inflammatory activity two protocols, so called, preventive and therapeutic, were used for *in vitro* assays. In the former, after 2 h of incubation, J774.1 cells were treated with different concentrations (0.5; 2; 8; 32 and 125 μg/mL) of plant extracts (100 μL/well) and incubated for additional 2 h. Then, 100 μL/well of a solution containing LPS (*Escherichia coli* 055: B5; Sigma, St. Louis, MO, USA) diluted in DMEM-c was added to the culture to obtain a final concentration of 0.5 μg/mL/well. After 22 h of LPS stimulation, the supernatant was collected and stored at −20 °C. In the therapeutic protocol cells were pre-stimulated with LPS (0.5 μg/mL) and, after 2 h were treated with different concentrations of plant extracts as described above. After 24 h of LPS stimulation, the supernatant was collected and stored at −20 °C. The supernatants were used for quantification of nitric oxide and cytokines.

2.3. In vivo experiments

The *in vivo* cytotoxicity and anti-inflammatory effects of the plant extract were assessed using *Mus musculus*, C57Bl/6 male and female mice weighing 20–25 g obtained from the Animal Facility of Faculdade de Ciências Farmacêuticas de Ribeirão Preto (FCFRP-USP). The animals were maintained on 12/12 h light/dark cycles with water and food *ad libitum* at temperatures of 21 ± 1 °C at the Animal Facility. All procedures were performed in accordance with ethical standards of Institutional Animal Care and Use Committee (protocol number 15.1.861.60.6), and executed according to the criteria outlined by the Brazilian Society for Laboratory Animal Science (SBCAL).

2.4. Single-dose assessment of in vivo toxicity

To address acute toxicity *in vivo,* mice were orally treated with a single dose of vehicle or the plant extract in different concentrations as follows: control group treated with saline only (Vehicle); and three groups treated with the plant extract at 1000 mg/kg and 5000 mg/kg body weight (b.w.). Every 2 days, mice were evaluated for weight variation, water consumption and individual food intake. After 14 days of the single administration of vehicle or plant extract, mice were euthanized in a carbon dioxide chamber. Then, liver was removed, macroscopically analyzed, and stored.

2.5. Repeated-dose assessment of in vivo toxicity

Repeated dose toxicity was determined in males and females mice treated by gavage twice a day (every 12 h) during 14 days with filtered water or the plant extract at 300 mg/kg or 1000 mg/kg b.w. Daily macroscopic evaluation was performed with observation of modifications of the following parameters: skin and hair texture; secretions in the eyes and mucous membranes; changes in respiration, motor system,

muscle tone and central nervous system (tremors, convulsion and sedation) and autonomic nervous system (lacrimation, salivation) as standardized by the Brazilian Health Regulatory Agency (ANVISA). Then, mice were euthanized in a carbon dioxide chamber and blood samples were collected in heparinized tubes for determination of Alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

2.6. Nitrite quantification

The amount of nitrite present in the cell culture supernatants and in the air pouch wash was determined as an indicator of NO production (Green et al., 1981). The total amount of nitrite in samples was calculated based on the absorbance of the serial dilution of $NaNO_2$ standard curve. The absorbance was measured at 540 nm in spectrophotometer (Bio-Tek Instruments, Inc. - Quant, Winooski, VT, USA).

2.7. Cytokine quantification

The concentration of TNF-α, IL-6, IL-1β and IL-10 in culture supernatants and in the air pouch wash was quantified by ELISA according to the manufacturers' instructions (R & D Systems, Minneapolis, USA). The optical densities were measured at 405 nm in a microplate reader (Bio-Tek Instruments, Inc. - Quant, Winooski, VT, USA). The results were expressed in pg/mL. Sensitivity was ≤10 pg/mL.

2.8. NF-κB reporter assay

RAW-Blue™ macrophages stably express the secreted embryonic alkaline phosphatase (SEAP) gene, which is inducible by the NF-κB/AP-1 transcription factors were donated by Dr. Huy Ong (Université de Montréal, Canada). The cells were seeded in 96-well micro culture plates at a density of 2×10^5 cells/well in DMEM supplemented with Normocin™ (50 mg/mL) and cultured at 37 °C in a humidified 5% CO_2 atmosphere for 18 h. After this period, according to the preventive protocol, cells were incubated with different concentrations (0.5; 2; 8; 32 and 125 μg/mL) of plant extracts (100 μL/well) and incubated again at 37 °C 5% CO_2. After 2 h, 100 μL/well of LPS-containing medium (0.5 μg/mL) was added as previously described in section 2.2. For therapeutic protocol, the cells were incubated with 100 μL/well of LPS-containing medium (0.5 μg/mL). After 2 h, cells were treated with the aforementioned concentrations of the plant extracts (100 μL/well) and incubated at 37 °C, 5% of CO_2 for 24 h as previously described in section 2.2. Then, the supernatants were collected, and 50 μL were mixed with 150 μL of QUANTI-Blue™ (InvivoGen, San Diego, CA, USA), which is a SEAP detection medium in 96-well plates at 37 °C for 2 h. The optical density was then measured at 650 nm using an ELISA reader (Bio-Tek Instruments, Inc. - Quant, Winooski, VT, USA).

2.9. Air-pouch

Prior to the induction of air-pouch mice were anesthetized with ketamine and xilasin (75 and 10 mg/kg, respectively). Air-pouch was induced in the dorsal region by subcutaneous injection of 3 mL of sterile air on days 0 and 3.

On day 6, mice received the stimulus and treatments (n = 5 mice/group) in the air-pouch (Fronza et al., 2016), and were arranged in different groups as follows: mice without inflammatory stimulus treated with 1 mL of PBS and 1 h later another 1 mL of PBS (group 1); mice inoculated with LPS, received 1 mL of LPS (0.5 μg/mL) and 1 h later 1 mL of PBS (group 2); mice treated with plant extract without inflammatory stimuli, received 1 mL of plant extract at 300 mg/kg and 1 h later 1 mL of PBS (group 3); mice inoculated with 1 mL of LPS (0.5 μg/mL) and 1 h later treated with 1 mL of plant extract at a concentration of 5 mg/kg (group 4); mice inoculated with 1 mL of LPS (0.5 μg/mL) and 1 h later treated with 1 mL of plant extract at 60 mg/kg (group 5); mice inoculated with 1 mL of LPS (0.5 μg/mL) and 1 h later 1 mL of plant extract at 300 mg/kg (group 6).

After 4 h of the last injection, mice were euthanized in CO_2 chamber and the air-pouch content was washed with 2 mL heparinized PBS (10 IU/mL). The exudates were collected with a pipette through an incision in the air pouch. Total and differential cell count, proteins and cytokines concentration, were determined in the exudates as previously described (Fronza et al., 2016).

2.10. Determination of protein concentration in air pouch wash

The air pouch washes were centrifuged at 1000 *g* for 15 min at 4 °C, and the supernatants used to quantify proteins, employing Bradford's assay (Bradford, 1976). Briefly, in a 96-well plate, 10 μL of supernatants were added to 200 μL of Bradford's reagent. After 10 min, the absorbance was checked at 595 nm in spectrophotometer (Bio-Tek Instruments, Inc. - Quant, Winooski, VT, USA). The results were compared to a standard curve with bovine serum albumin, with concentrations ranging from 50 to 300 ng/mL.

2.11. Statistical analyses

The data represent the mean ± SEM. The variants were determined by Student's test-t and One-Way ANOVA Tukey post hoc ANOVA. Values of $p < 0.05$ were considered significant. All analyzes were performed using GraphPad Prism 7.0.

3. Results

3.1. Ethanolic extract of Terminalia argentea is not toxic

First, to address the *in vitro* toxicity of the ethanolic extract of *T. argentea,* J 774.1 macrophages were cultivated with different concentrations of the extract as described in *Methods and Materials*. Regardless the concentration of the extract used no alterations were observed in cell viability (Fig. 1A). Because no deleterious effect was observed *in vitro*, we aimed to elucidate if the plant extract had some toxicity *in vivo*. Thus, mice were orally treated with a single dose of vehicle (saline), 1000 or 5000 mg/kg of plant extract and followed by 14 days until euthanasia. Treatment had no effect on water and food consumption (data not shown). In addition, treatment seemed not to impact the function of hepatic transaminases AST and ALT even when the highest single dose was used (Fig. 1B and C, respectively). As no toxicity was observed after the administration of a high single dose, we explored the effects of twice-daily consumption of lower to intermediate doses of the plant extract. Regardless if 300 mg/kg or 1000 mg/kg was used twice daily for 14 days, no signs of toxicity, including weight loss, reduction in water or food consumption, higher production of hepatic aminotransferases were observed (data not shown). Altogether, these results suggest the ethanolic extract of *T. argentea* is not toxic either when tested *in vitro* or *in vivo*, regardless if it is used once in higher doses or daily for longer periods in lower concentrations.

3.2. Ethanolic extract of Terminalia argentea inhibits the release of nitric oxide and modulates the production of cytokines in LPS-stimulated macrophages

Because of the importance of modulating NO and cytokines to constrain the onset and development of inflammation the *in vitro* effects of the ethanolic extract of *T. argentea* towards the production of such molecules was assessed in macrophages stimulated with LPS. Thus, LPS-stimulated J774.1 macrophages were treated with different concentrations of the extract. Further, to explore both preventive and therapeutic properties within molecules in the plant extract two protocols were used. Regardless the concentration of plant extract and protocol used, a reduction in the production of NO was observed (Fig. 2A). Similarly, when compared to macrophages treated with LPS only, a reduction was

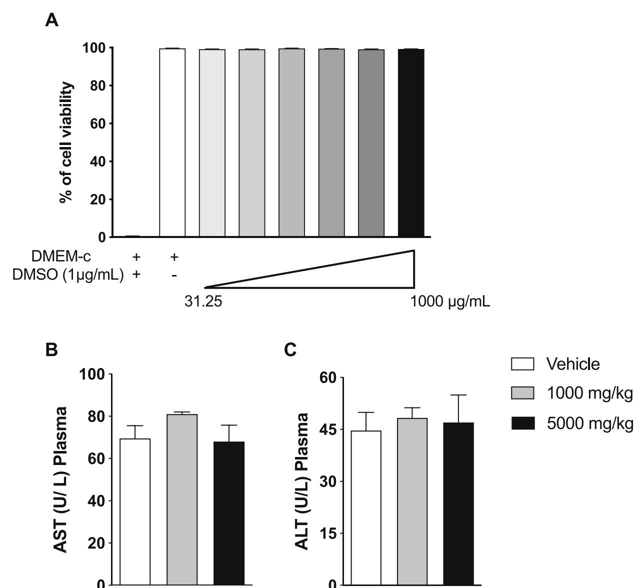

Fig. 1. Ethanolic extract of *Terminalia argentea* Mart. & Zucc. showed no in vitro or in vivo toxicity. (A) J 774.1 cells were treated with DMSO (1 μg/mL), medium or the extract at 31.25, 62.5, 125, 250, 500 and 1000 μg/mL for 24 h. Cell viability was measured by MTT assay. Mice (n = 3) received water (vehicle) or a single dose of *T. argentea* extract at 1000 mg/kg or 5000 mg/kg. They were followed for 14 days and euthanized for plasma collection, which was used to quantify (B) aspartate aminotransferase (AST) and (C) alanine aminotransferase (ALT). U/L: units per litter.

observed in the production of TNF-α and IL-6 in cells treated with different concentrations of plant extract (Fig. 2B and C, respectively). The ability of *T. argentea* extract to restrain the production of inflammatory cytokines in LPS-stimulated macrophages was reinforced when the levels of IL-1β were assessed. However, this effect seemed to be more dose-dependent since the highest concentrations induced greater inhibition of IL-1β production, regardless the protocol used to treat macrophages *in vitro* (Fig. 2D). Despite the importance of reducing the production of inflammatory cytokines to constrain inflammation, therapies aiming also at improving the levels of anti-inflammatory cytokines, such as IL-10, tend to produce more promising results. In this regard, treatment with the plant extract, especially when used at the highest concentration and in therapeutic protocol, was able to induce the production of IL-10 by macrophages when compared to cells treated with LPS only (Fig. 2E). Taken together, these results suggest the ethanolic extract of *T. argentea* is able to modulate key aspects to control inflammation regardless the protocol used.

3.3. The ethanolic extract of *Terminalia argentea* inhibits the activation of the transcription factor NF-κB

Next, we aimed to elucidate the mechanisms concerning the activity of the plant extract towards the production of inflammatory mediators. First, we addressed the role of plant extract in the absence of inflammatory stimuli. Thus, in a dose-dependent manner treatment using the ethanolic plant extract was able to inhibit the activity of NF-κB (Fig. 3A), even in the absence of inflammatory stimuli. Regardless the protocol used to treat cells, plant extract reduced the activity of NF-kB in a dose dependent manner (Fig. 3B and C). The utmost inhibition of NF-κB activity was observed when the highest concentrations of plant extract were used. These results suggest that *T. argentea* extract constrain the production of inflammatory mediators by the negative modulation of NF-κB.

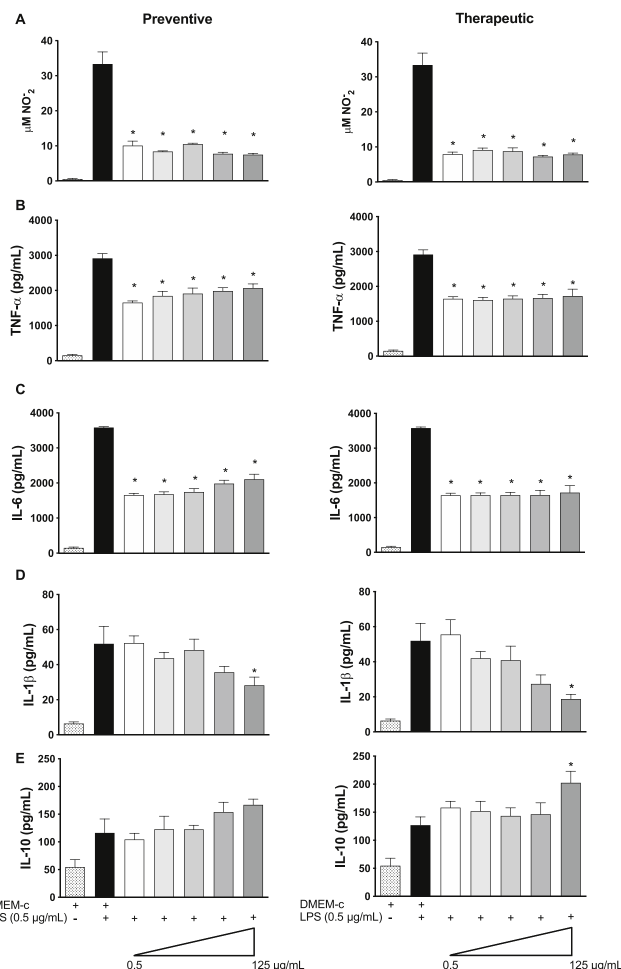

Fig. 2. The ethanolic extract of *Terminalia argentea* Mart. & Zucc. modulates key aspects to constrain inflammation. J 774.1 cells were treated with medium (DMEM-c), LPS (0.5 μg/mL), and/or with the extract at 0.5, 2, 8, 32 and 125 μg/mL for 24 h. The production of nitric oxide (A) was estimated by the concentration of nitrites in culture supernatants. The production of the cytokines(B) TNF-α, (C) IL-6, (D) IL-1β and (E) IL-10 in culture supernatants was assessed by ELISA. Right column: cells treated with the ethanolic extract at different concentrations and 2 h later with LPS (Preventive). Left column: cells incubated with LPS and 2 h later treated with different concentrations of the plant extract (Therapeutic). * comparison between LPS only- and plant extract-treated cells ($p < 0.05$).

3.4. *Terminalia argentea* extract modulates the production of key players in LPS-induced inflammation and the number of leukocytes in the air pouch

Because *T. argentea* extract was able to modulate the production of pro- and anti-inflammatory mediators in macrophages, besides of not inducing toxicity, we aimed to elucidate if the plant extract was able to modulate inflammation in mice. The model of inflammation used was the air pouch model inoculated with LPS. Thus, treatment with *T. argentea* extract was able to reduce the production of total proteins in the air pouch environment after stimulation with LPS, especially when the concentration of 300 mg/kg was used, when compared to the other concentrations (5 and 60 mg/kg) or vehicle (Fig. 4A). Next, we aimed to evaluate the effects of the plant extract on recruited leukocytes to the site of inflammation. In accordance with the reduction of total proteins in the air pouch, plant extract at the highest concentration (300 mg/kg), reduced the number of neutrophils and mononuclear cells (Fig. 4B and C, respectively) in the same location. To a less extent, a reduction in the number of neutrophils was observed in mice treated with 5 and 60 mg/

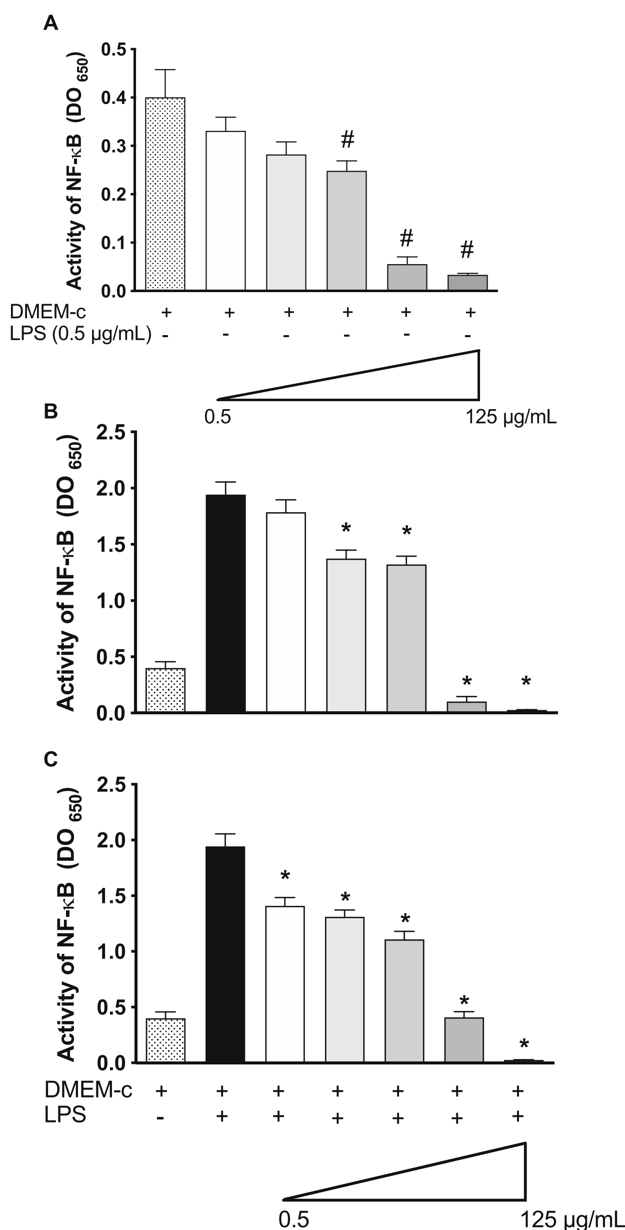

Fig. 3. The anti-inflammatory activity of the ethanolic extract of *Terminalia argentea* Mart. & Zucc. relies on the supression of NF-κB activity. RAW-Blue™ macrophages were incubated during 24 h in 96-well plate with or without the inflammatory stimuli LPS and ethanolic extract. (A) cells were incubated in the absence of LPS with 0.5, 2, 8, 32 and 125 μg/mL of ethanolic extract of *T. argentea*; (B) cells were treated with the aforementioned concentrations of the ethanolic extract of *T. argentea* 2 h prior stimulation with LPS (0.5 μg/mL) (preventive protocol); (C) cells were treated with LPS and 2 h later incubated with the ethanolic extract of *T. argentea* at different concentrations. Then, the supernatants were collected to evaluate the activation of NF-κB. The QUANTI-Blue ™ substrate was used to measure the secreted embryonic alkaline phosphatase (SEAP) at 650 nm in a spectrophotometer. Differences were considered significant when $P < 0.05$. # medium versus extracts and *LPS only vs LPS + extracts.

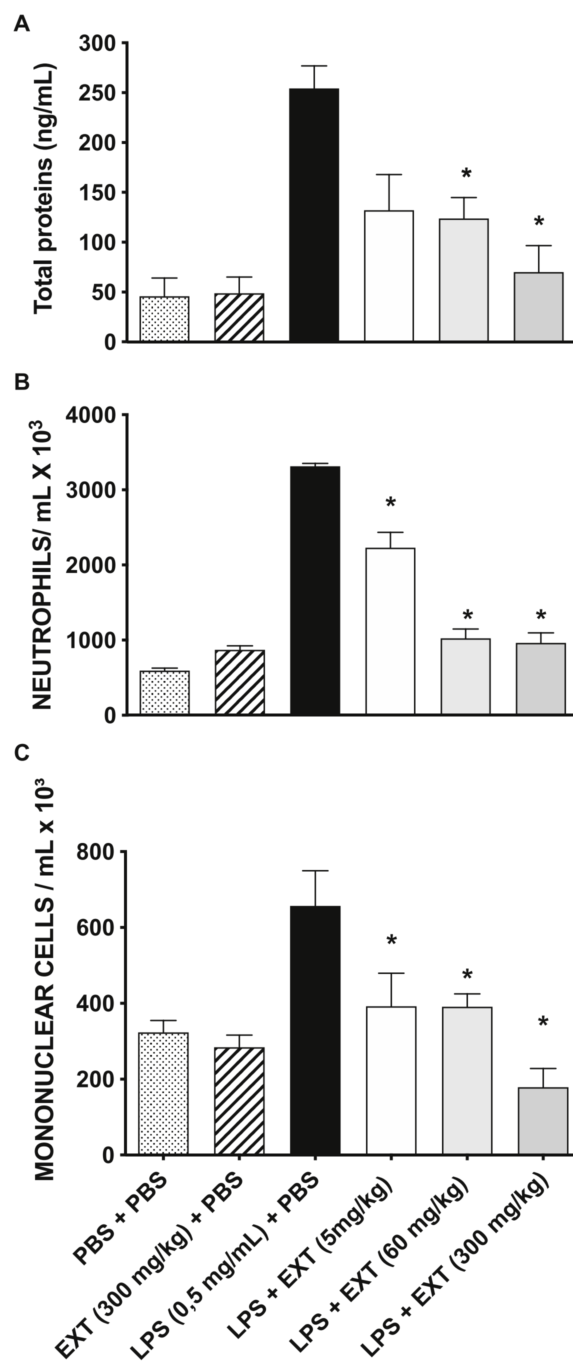

Fig. 4. The *Terminalia argentea* Mart. & Zucc. extract modulates cell migration and protein production during inflammation. Air pouch was induced in C57BL/6 mice (n = 3–5 mice/group) and the groups were arranged as follows: Phosphate buffered saline (PBS) + PBS; PBS + ethanolic extract of *T. argentea* (300 mg/kg) (EXT + PBS); LPS (0.5 μg/mL) + PBS (LPS + PBS); LPS (0.5 μg/mL) and 1 h later the extracts (LPS + EXT) at the concentrations of 5, 60 and 300 mg/kg. After 4 h of the treatment onset, mice were euthanized and the air pouches washed with heparinized-PBS and centrifuged. (A) Protein concentration was determined by the Bradford reaction. Cellular recruitment was determined by total and differential count of neutrophils (B) and mononuclear cells (C). *$p < 0.05$ LPS + PBS versus LPS + EXT.

kg of the plant extract, when compared to those receiving only the vehicle (Fig. 4B).

As we observed the anti-inflammatory effect of the plant extract in air pouch during LPS-induced inflammation, our next step was to quantify the cytokines locally produced. Treatment with 300 mg/kg of plant extract induced a reduction in IL-6 (Fig. 5A), TNF-α (Fig. 5B) and IL-1β (Fig. 5C) along with increased concentration of the anti-inflammatory cytokine IL-10 (Fig. 5D), when compared to air-pouch stimulated with LPS and treated with PBS only. Local treatment in the air pouch using 60 mg/kg also demonstrated immunomodulatory properties, especially by reducing TNF-α (Fig. 5B) and augmenting IL-10 (Fig. 5D), when compared to mice locally treated with PBS only.

However, no beneficial effects were observed when the lowest concentration of the plant extract was used to treat inflammation in the air pouch.

4. Discussion

The results presented here demonstrate the anti-inflammatory properties of the ethanolic extract of *T. argentea*. Furthermore, regardless the concentration or the experimental approach used, plant extract showed no toxicity nor deleterious effects on cell lineage or liver. One of the leading concerns regarding the use of natural products relies on their impact on organ functionality. A previous study have shown the hepatoprotective effect of a different member of family Combretaceae, known as *T. arjuna* (Doorika and Ananthi, 2012). This beneficial effect was observed when the aqueous plant extract was given orally at 200 mg/kg for 20 days to rats. Treatment reduced biochemical markers including AST and ALT, after induction of hepatotoxicity by using Isoniazid (100 mg/kg) for 10 days (Doorika and Ananthi, 2012). In addition, the methanolic extract of *T. arjuna* bark also showed a gastroprotective effect when administered at 400 mg/kg in rats (Devi et al., 2007a, 2007b). The aqueous extract of *T. paniculata* bark at 2000 mg/kg, was not toxic nor induced death in rats, besides of presenting significant hypoglycemic, hypolipidemic and antioxidant activities after induction of diabetes with streptozotocin (Ramachandran et al., 2012). Despite the differences in experimental models, concentrations and plant species used in the aforementioned studies, the plant extract did not induce cell toxicity nor disturbed the production of liver transaminases in the present study. In addition, the concentration with the highest therapeutic activity used in our study (300 mg/kg) is also in accordance with others used with different species from the same family. To the best of our knowledge, our study explores for the first time the *in vitro* and *in vivo* toxicity of the hydroalcoholic extract of *T. argentea* bark. However, further studies exploring long-term exposure to the plant extract must be conducted to clarify the occurrence of toxicity in this scenario.

In order to restrain inflammation, a therapeutic candidate must be able to modulate different players in this scenario. Among them, it is possible to highlight the roles of nitric oxide (NO) and cytokines, which are crucial to the onset and maintenance of inflammation and inflammatory diseases. Nitric oxide is an unstable and highly soluble molecule belonging to the family of reactive oxygen species (ROS), as reviewed elsewhere (Mijatovic et al., 2020). Despite its first description in promoting blood vessel relaxation (Gruetter et al., 1979), NO has been implicated in a variety of biological activities including the immune response and neurotransmission (Tewari et al., 2020). During inflammatory conditions, pro-inflammatory cytokines may induce the production of NO, especially in immune and non-immune cells. In general, mostly in the presence of infectious agents like bacteria and their derived products, such as LPS, the production of NO is drastically enhanced in phagocytes and other cells in the body, which improves phagocytosis and microbial destruction (Galkina et al., 2019). Nevertheless, overproduction of NO has been implicated in the pathogenesis of inflammatory conditions such as inflammatory bowel disease, rheumatoid arthritis and diabetes, among others (Luczak et al., 2020). This role, can be at least partly attributed to the effects of NO on enhancing the activity of both the constitutive and inducible forms of cyclooxygenase (COX) enzymes (McDaniel et al., 1996). This mechanism leads to the over production of proinflammatory mediators, prostaglandins, thus initiating and sustaining inflammation (McDaniel et al., 1996). Cytokines, especially IL-1β, can also enhance the production of NO or have its own production positively influenced by this molecule (Mijatovic et al., 2020). Thus, it seems clear that therapies aiming at modulating the production of NO may represent important strategies to control inflammation. Although we have only addressed the *in vitro* effects of the plant extract in the downregulation of NO, we cannot underestimate its participation in constraining the inflammation induced in mice nor its influence in the reduction of inflammatory cytokines.

The production of inflammatory cytokines is a complex process dependent on the activation of specific receptors, pathways, proteins and ultimately nuclear transcription factors. The NF-κB is one of the major nuclear factors involved in the control of apoptosis, cell proliferation, stress responses, cancer progression and the activation of pro-inflammatory genes, thus leading to the transcription of inflammatory mediators such as IL-1β, TNF-α, IL-6 IL-8, iNOS and COX-2, as previously reviewed (Tsaouli et al., 2020). Therefore, because of its central role in controlling the onset and maintenance of inflammation it is also related to the control of innate and adaptive immune responses. The deregulated activity of NF-κB has been investigated in diabetes, inflammatory bowel disease, hypertension, ischemia, neoplastic malignancies and other disorders (Coto et al., 2018; House et al., 2017; Lee et al., 2018; Pang et al., 2018; Ye et al., 2018). Thus, it is feasible to assume that drugs targeting the inhibition of NF-κB activity may represent promising candidates to treat human illnesses. In accordance to the effects observed in the present study with *T. argentea*, different researchers have shown the ability of other members of the family Combretaceae plants to downregulate the activity of NF-κB. Chebulic acid, which is one of the main compounds isolated from the ethanolic extract of *T. chebula* fruits, was able to reduce the release of LPS-induced mediators by suppressing NF-κB activity in RAW 264.7 macrophages (Reddy and Reddanna, 2009). This modulation was followed by the inhibition in the expression of iNOS, COX-2, 5-lipoxygenase (5-LOX), TNF-α and IL-6, along with a reduction in the production of NO and PGE_2 (Reddy and Reddanna, 2009), which are stronger inducers of inflammation. Similarly, but using an aqueous extract of *T. chebula* fruit at 50 μg/mL, a suppression in NF-κB functionality was also observed in human lymphoblastic T cells (Das et al., 2011). Altogether, these studies suggest the suppression of NF-κB as a central mechanism in the activity of some Combretaceae plant extracts, likewise the results observed in the present study. Despite the fact that the composition of the ethanolic extract of *T. argentea* is already known, we have not identified which molecule is related to the suppression of NF-κB activity observed in our study.

The mouse air-pouch is considered to be a good model to study acute inflammation induced by a variety of agents including lipid mediators and LPS (Medeiros et al., 2015; Segawa et al., 2016). As expected, the inflammatory stimuli either *in vitro* or *in vivo* induced, in the present study, the production of the inflammatory cytokines IL-6, IL-1β and TNF-α, besides of elevated levels of total proteins, neutrophils and mononuclear cells. On the other hand, the inflammatory stimuli also reduced the production of anti-inflammatory mediators such as IL-10. All these results are in accordance with the onset of inflammation and the immune imbalance observed in such situation. In this context, treatment using the ethanolic extract of *T. argentea* bark was able to reestablish the immune balance regardless if it was used in *in vitro* or *in vivo* experiments.

IL-6 is a pleiotropic cytokine produced by different type of cells which expression is strongly stimulated by IL-1β and TNF-α (Hodge et al., 2005). Additionally, IL-6 is directly associated to hematopoiesis, activation and recruitment of neutrophils to inflammatory sites besides of being related to the induction and differentiation of Th17 cell subtype and the control of B cells and humoral immunity, as previously reviewed (Liu, 2020). Thus, it is clear the role of IL-6 in modulating both innate and adaptive immune responses, which makes this molecule a crucial target to therapies aiming at controlling inflammation. Some other studies have explored the ability of different members of family Combretaceae plant extracts to constrain inflammation by reducing the levels of IL-6. This was observed with extracts from *T. chebula* Retz. in RAW 264.7 macrophages (Reddy and Reddanna, 2009), and *in vivo* both in a murine model of arthritis (Nair et al., 2010) and after induction of acute liver injury in mice (Choi et al., 2015). Similarly, the ethanolic extract of *T. catappa* leaves also induced a reduction in IL-6 in liver of mice with carbon tetrachloride-induced hepatotoxicity (Gao et al., 2004). Despite the differences in experimental approaches, doses and the chemical isolation of the extracts, to the best of our knowledge, our

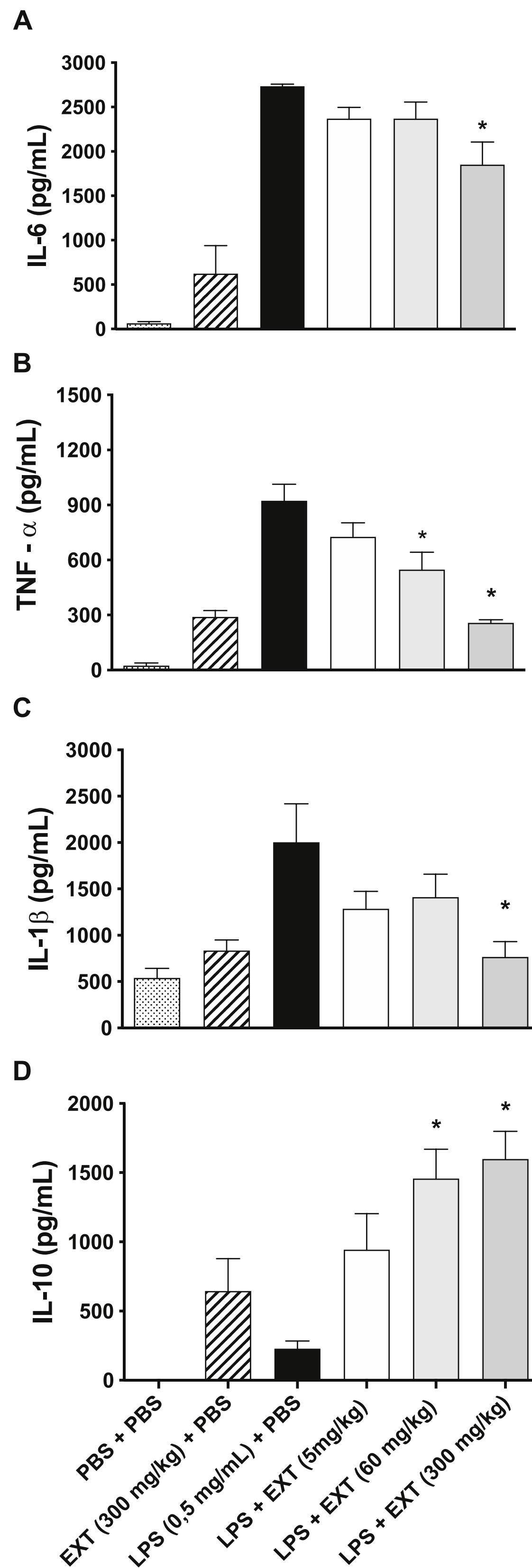

Fig. 5. The *Terminalia argentea* Mart. & Zucc. extract modulates the production of cytokines during inflammation. Air pouch was induced in C57BL/6 mice (n = 3–5 mice/group) and the groups were arranged as follows: Phosphate buffered saline (PBS) + PBS; PBS + ethanolic extract of *T. argentea* (300 mg/kg) (EXT + PBS); LPS (0.5 μg/mL) + PBS (LPS + PBS); LPS (0.5 μg/mL) and 1 h later the extracts (LPS + EXT) at the concentrations of 5, 60 and 300 mg/kg. After 4 h of the treatment onset, mice were euthanized and the air pouches washed with heparinized-PBS and centrifuged. Then, the production of IL-6 (A), TNF-α (B), IL-1β (C) and IL-10 was determinate by ELISA. *$p < 0.05$ LPS + PBS versus LPS + EXT.

study is the first to address the role of the ethanolic extract of *T. argentea* bark on the reduction of IL-6.

TNF-α is another key player in the development of inflammation and immune response against infectious agents (So and Ishii, 2019). It is primarily produced by activated macrophages and lymphocytes and directly influence the differentiation of monocytes into macrophages and recruitment of neutrophils to granulomas (Kindler et al., 1989). Because of the importance of this cytokine to the onset and maintenance of inflammation, it has been used as one of the most important targets to treat immune-mediated diseases. The pharmacological inhibition of TNF-α reduces the activity of inflammatory mediators such as NO and VEGF (Campanati et al., 2012), besides of reducing the production of IL-17 and the number of Th17 lymphocytes (Piaserico et al., 2014). In this context, pre-treatment for 16 h with *T. arjuna stem-bark* extract was able to reduce the production of TNF-α in the cell lineage of monocytic leukemia cells – THP-1 (Kokkiripati et al., 2013). Though pre-treatment using both ethanolic and aqueous extract of *T. arjuna* stem-bark was able to reduce the production of TNF-α after stimulation with LPS, the inhibition observed with the former was greater than that observed with the later (Kokkiripati et al., 2013). The anti-inflammatory activity of the ethanolic extract of *T. arjuna* bark was reinforced in an experimental model of chronic heart failure induced with isoproterenol in rats (Parveen et al., 2011). Oral treatment prior and 15 days after heart failure induction, was able to attenuate cardiac dysfunction besides of drastically reducing the levels of TNF-α in peripheral blood (Parveen et al., 2011). Chebulagic acid, which is isolated from the ethanolic extract of *T. chebula* fruits, inhibited the production of TNF-α and other important inflammatory mediators in RAW 264.7 macrophages stimulated with LPS (Reddy and Reddanna, 2009). The immunomodulatory and anti-arthritic properties of the ethanolic extract of *T. chebula* were at least partly attributed to the reduction of TNF-α in serum of diseased rats (Nair et al., 2010). Altogether, these results suggest the higher efficacy of ethanolic extracts of different Terminalia' members to constrain the production of TNF-α. Though, in the present study, we have not explored the effects of the ethanolic extract of *T. argentea* in other inflammatory conditions, our results are in accordance with other studies regarding the effects of the plant extract in diminishing the production of TNF-α. Furthermore, this is the first description of the effects of the ethanolic extract of *T. argentea* towards TNF-α production.

IL-1 is a cytokine involved in different aspects of immune response including the induction of fever, neutrophil influx and activation of T- and B-cells, besides of production of prostaglandin and cytokines, as reviewed elsewhere (Christgen et al., 2020). This cytokine is produced by two distinct genes known as IL-1α and IL-1β. The latter, is available as an inactive pro-form and needs to be cleaved before it is biologically available (Agostini et al., 2004). IL-1β is preferentially produced by monocytes, macrophages, dendritic cells, B-lymphocytes and natural killer cells (Agostini et al., 2004; Netea et al., 2009). Additionally, local production of IL-1β is related to neutrophil recruitment (Patton et al., 1995), differentiation of Th17 lymphocytes (Aliahmadi et al., 2009), and lung edema (Zoccal et al., 2016). These data strongly suggest a central role for IL-1β in the promotion of inflammation and cell recruitment. Thus, therapies aiming at modulating this cytokine can also contribute to the control of inflammatory illnesses. In this context, an Indian herbal formulation, known as *Triphala*, which contains *T. chebula*, *T. bellirica* (Gaertn.) Roxb. and *Emblica officinalis* Gaertn., was shown to be effective to treat arthritis induced in rats (Kalaiselvan and Rasool, 2015). The amelioration in clinical outcome was directly associated to a reduction in serum and paw tissues of inflammatory mediators such as TNF-α, IL-1β, VEGF, MCP-1, and PGE_2 (Kalaiselvan and Rasool, 2015). In addition, IL-1β was the molecule more affected, either systemically or locally, by the therapy using the herbal formulation. Despite the fact that we have not explored the role of the ethanolic extract of *T. argentea* bark in the modulation of VEGF, MCP-1, and PGE_2 neither explored its anti-inflammatory activity in combination with other plant extracts, our study highlighted for the first time the immunomodulatory properties of the ethanolic extract of *T. argentea* bark both in *vitro* and *in vivo*. Furthermore, because of the role of IL-6, TNF-α and IL-1 β in cell activation and recruitment, we hypothesized this modulation may also have influenced the reduction in the levels of circulating neutrophils and mononuclear cells observed in the present study.

The immune balance is tightly regulated by pro- and anti-inflammatory mediators. In this scenario, IL-10 is one of the most studied anti-inflammatory cytokines with a crucial role in preventing immune-mediated diseases (Fiorentino et al., 1991; Grunig et al., 1997). IL-10 is produced by different cells of the immune system both from innate and adaptive immunity. This cytokine mediates its anti-inflammatory activity directly or indirectly suppressing the immune responses by inhibiting T cell activation; TLR-signaling in antigen presenting cells (APC) and APC functionality and antigen presentation, among other mechanisms (Kany et al., 2019). The combination of chebulagic acid and punicalagin, both obtained from the extract of *T. chebula* fruits, was able to attenuate the inflammation induced in respiratory tract after infection with respiratory syncytial virus BALB/c mice (Xie, 2016). Mice treated with the molecules had reduced viral-induced lung lesions and production of inflammatory mediators such as iNOS, COX-2, PGE_2, IL-6, TNF-α and IL-1β in lung along with an improvement in the local production of IL-10 (Xie, 2016). The ability of compounds obtained from Terminalia' plants to induce the production of IL-10 was reinforced in a different study. Arjunolic acid, isolated from *T. arjuna* bark, was able to reduce, after 6 months of treatment, the circulating levels of IL-6, IL-18, TNF-α and c-reactive protein in patients with stable coronary artery disease (Kapoor et al., 2015). This amelioration, was also associated to the elevation in serum levels of IL-10 (Kapoor et al., 2015). Taken together, despite the differences in experimental models and species of *Terminalia* used, these data are in accordance with those shown in the present study.

5. Conclusions

In conclusion, our results showed for the first time the anti-inflammatory and immunomodulatory properties of the ethanolic extract of *T. argentea* bark. The extract was able to constrain the release of inflammatory mediators *in vitro* and the development of inflammation *in vivo*, and reestablish the immune balance with no signals of toxicity. Further, the mechanism of action seems to be at least partly dependent on downregulation of NF-κB. Finally, our results also suggest the ethanolic extract of *T. argentea* bark as a promising candidate to treat immune-mediated diseases.

Authors contributions

Mirella dos Reis de Araújo Moreira, Helioswilton Sales-Campos, Milton Groppo Junior and Lúcia Helena Faccioli designed the study. Mirella dos Reis de Araújo Moreira, Helioswilton Sales-Campos, Caroline Fontanari, Alyne Fávero Galvão Meireles, Morgana Kelly Borges Prado, Karina Furlani Zoccal, Carlos Artério Sorgi and Cristiane Tefé da Silva performed experiments and were involved with data collection and analysis. Carlos Artério Sorgi, Cristiane Tefé da Silva and Milton Groppo critically reviewed the manuscript. Milton Groppo identified the plant material and deposited the vouchers in the herbarium. Mirella dos Reis de Araújo Moreira, Helioswilton Sales-Campos and Lúcia Helena Faccioli wrote the manuscript. All authors have read and approved the final version of the manuscript.

Funding sources

This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP grant #2014/07125–6 and #2014/03332–7 to KFZ; #2006/06260–2 to MG), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, grant n. 302514/2015–5) and Programa Nacional de Pós-Doutorado (PNPD) -

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) to HSC.

Declaration of competing interest

The authors have no conflict of interest to declare.

List of Abbreviations

ALT	alanine aminotransferase
AST	aspartate aminotransferase
ANVISA	Brazilian Health Regulatory Agency
ATCC	American Type Culture Collection
b.w	body weight
FCFRP	Faculdade de Ciências Farmacêuticas de Ribeirão Preto
LPS	liposaccharide
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NO	nitric oxide
PBS	Phosphate buffer saline
ROS	reactive oxygen species
SBCAL	Brazilian Society for Laboratory Animal Science
SEAP	secreted embryonic alkaline phosphatase

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jep.2020.113150.

References

Agostini, L., Martinon, F., Burns, K., McDermott, M.F., Hawkins, P.N., Tschopp, J., 2004. NALP3 forms an IL-1beta-processing inflammasome with increased activity in Muckle-Wells autoinflammatory disorder. Immunity 20 (3), 319–325.

Aliahmadi, E., Gramlich, R., Grutzkau, A., Hitzler, M., Kruger, M., Baumgrass, R., Schreiner, M., Wittig, B., Wanner, R., Peiser, M., 2009. TLR2-activated human langerhans cells promote Th17 polarization via IL-1beta, TGF-beta and IL-23. Eur. J. Immunol. 39 (5), 1221–1230.

Allouche, Y., Jimenez, A., Uceda, M., Aguilera, M.P., Gaforio, J.J., Beltran, G., 2009. Triterpenic content and chemometric analysis of virgin olive oils from forty olive cultivars. J. Agric. Food Chem. 57 (9), 3604–3610.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Campanati, A., Orciani, M., Gorbi, S., Regoli, F., Di Primio, R., Offidani, A., 2012. Effect of biologic therapies targeting tumour necrosis factor-alpha on cutaneous mesenchymal stem cells in psoriasis. Br. J. Dermatol. 167 (1), 68–76.

Choi, M.K., Kim, H.G., Han, J.M., Lee, J.S., Lee, J.S., Chung, S.H., Son, C.G., 2015. Hepatoprotective effect of Terminalia chebula against t-BHP-induced acute liver injury in C57/BL6 mice. Evid. based Compl. Alternat. Med. 517350, 2015.

Christgen, S., Place, D.E., Kanneganti, T.D., 2020. Toward targeting inflammasomes: insights into their regulation and activation. Cell Res. 30 (4), 315–327.

Coto, E., Diaz-Corte, C., Tranche, S., Gomez, J., Alonso, B., Iglesias, S., Reguero, J.R., Lopez-Larrea, C., Coto-Segura, P., 2018. Gene variants in the NF-KB pathway (NFKB1, NFKBIA, NFKBIZ) and their association with type 2 diabetes and impaired renal function. Hum. Immunol. 79 (6), 494–498. https://doi.org/10.1016/j.humimm.2018.03.008.

Das, N.D., Jung, K.H., Park, J.H., Mondol, M.A., Shin, H.J., Lee, H.S., Park, K.S., Choi, M.R., Kim, K.S., Kim, M.S., Lee, S.R., Chai, Y.G., 2011. Terminalia chebula extract acts as a potential NF-kappaB inhibitor in human lymphoblastic T cells. Phytother Res. 25 (6), 927–934.

Devi, R.S., Narayan, S., Vani, G., Shyamala Devi, C.S., 2007a. Gastroprotective effect of Terminalia arjuna bark on diclofenac sodium induced gastric ulcer. Chem. Biol. Interact. 167 (1), 71–83.

Devi, R.S., Narayan, S., Vani, G., Srinivasan, P., Mohan, K.V., Sabitha, K.E., Devi, C.S., 2007b. Ulcer protective effect of Terminalia arjuna on gastric mucosal defensive mechanism in experimental rats. Phytother Res. 21 (8), 762–767.

Doorika, P., Ananthi, T., 2012. Antioxidant and hepatoprotective properties of Terminalia arjuna bark on isoniazid induced toxicity in albino rats. Asian J. Pharm. Tech. 2 (1), 15–18.

During, A., Debouche, C., Raas, T., Larondelle, Y., 2012. Among plant lignans, pinoresinol has the strongest antiinflammatory properties in human intestinal Caco-2 cells. J. Nutr. 142 (10), 1798–1805.

Fahmy, N., Al-Sayed, E., Singab, A., 2015. Genus Terminalia: a phytochemical and biological review. Montin.) species. Med. Aromatic Plants 4, 218.

Fiorentino, D.F., Zlotnik, A., Mosmann, T.R., Howard, M., O'Garra, A., 1991. IL-10 inhibits cytokine production by activated macrophages. J. Immunol. 147 (11), 3815–3822.

Fronza, M., Muhr, C., da Silveira, D.S., Sorgi, C.A., Rodrigues, S.F., Farsky, S.H., Paula-Silva, F.W., Merfort, I., Faccioli, L.H., 2016. Hyaluronidase decreases neutrophils infiltration to the inflammatory site. Inflamm. Res. 65 (7), 533–542.

Galkina, S.I., Golenkina, E.A., Viryasova, G.M., Romanova, Y.M., Sud'ina, G.F., 2019. Nitric oxide in life and death of neutrophils. Curr. Med. Chem. 26 (31), 5764–5780.

Gao, J., Dou, H., Tang, X.H., Xu, L.Z., Fan, Y.M., Zhao, X.N., 2004. Inhibitory effect of TCCE on CCl4-induced overexpression of IL-6 in acute liver injury. Acta Biochim. Biophys. Sin. 36 (11), 767–772.

Green, L.C., Ruiz de Luzuriaga, K., Wagner, D.A., Rand, W., Istfan, N., Young, V.R., Tannenbaum, S.R., 1981. Nitrate biosynthesis in man. Proc. Natl. Acad. Sci. U. S. A. 78 (12), 7764–7768.

Gruetter, C.A., Barry, B.K., McNamara, D.B., Gruetter, D.Y., Kadowitz, P.J., Ignarro, L., 1979. Relaxation of bovine coronary artery and activation of coronary arterial guanylate cyclase by nitric oxide, nitroprusside and a carcinogenic nitrosoamine. J. Cyclic. Nucl. Res. 5 (3), 211–224.

Grunig, G., Corry, D.B., Leach, M.W., Seymour, B.W., Kurup, V.P., Rennick, D.M., 1997. Interleukin-10 is a natural suppressor of cytokine production and inflammation in a murine model of allergic bronchopulmonary aspergillosis. J. Exp. Med. 185 (6), 1089–1099.

Halder, S., Bharal, N., Mediratta, P.K., Kaur, I., Sharma, K.K., 2009. Anti-inflammatory, immunomodulatory and antinociceptive activity of Terminalia arjuna Roxb bark powder in mice and rats. Indian J. Exp. Biol. 47 (7), 577–583.

Hodge, D.R., Hurt, E.M., Farrar, W.L., 2005. The role of IL-6 and STAT3 in inflammation and cancer. Eur. J. Canc. 41 (16), 2502–2512.

House, C.D., Jordan, E., Hernandez, L., Ozaki, M., James, J.M., Kim, M., Kruhlak, M.J., Batchelor, E., Elloumi, F., Cam, M.C., Annunziata, C.M., 2017. NFkappaB promotes ovarian tumorigenesis via classical pathways that support proliferative cancer cells and alternative pathways that support ALDH(+) cancer stem-like cells. Canc. Res. 77 (24), 6927–6940.

Kalaiselvan, S., Rasool, M.K., 2015. The anti-inflammatory effect of triphala in arthritic-induced rats. Pharm. Biol. 53 (1), 51–60.

Kany, S., Vollrath, J.T., Relja, B., 2019. Cytokines in inflammatory disease. Int. J. Mol. Sci. 20 (23).

Kapoor, D., Trikha, D., Vijayvergiya, R., Parashar, K.K., Kaul, D., Dhawan, V., 2015. Short-term adjuvant therapy with Terminalia arjuna attenuates ongoing inflammation and immune imbalance in patients with stable coronary artery disease: in vitro and in vivo evidence. J. Cardiovasc. Transl. Res. 8 (3), 173–186.

Kindler, V., Sappino, A.P., Grau, G.E., Piguet, P.F., Vassalli, P., 1989. The inducing role of tumor necrosis factor in the development of bactericidal granulomas during BCG infection. Cell 56 (5), 731–740.

Kokkiripati, P.K., Kamsala, R.V., Bashyam, L., Manthapuram, N., Bitla, P., Peddada, V., Raghavendra, A.S., Tetali, S.D., 2013. Stem-bark of Terminalia arjuna attenuates human monocytic (THP-1) and aortic endothelial cell activation. J. Ethnopharmacol. 146 (2), 456–464.

Lee, S.H., Lee, H.R., Kwon, J.Y., Jung, K., Kim, S.Y., Cho, K.H., Choi, J., Lee, H.H., Lee, B.I., Jue, D.M., Cho, M.L., 2018. A20 ameliorates inflammatory bowel disease in mice via inhibiting NF-kappaB and STAT3 activation. Immunol. Lett. 198, 44–51.

Liu, A.Y., 2020. Infectious implications of interleukin-1, interleukin-6, and T helper type 2 inhibition. Infect. Dis. Clin. North. Am. 34 (2), 211–234. https://doi.org/10.1016/j.idc.2020.02.003.

Luczak, A., Madej, M., Kasprzyk, A., Doroszko, A., 2020. Role of the eNOS uncoupling and the nitric oxide metabolic pathway in the pathogenesis of autoimmune rheumatic diseases. Oxid. Med. Cell. Longev. 2020, 1417981.

McDaniel, M.L., Kwon, G., Hill, J.R., Marshall, C.A., Corbett, J.A., 1996. Cytokines and nitric oxide in islet inflammation and diabetes. Proc. Soc. Exp. Biol. Med. 211 (1), 24–32.

Medeiros, A.I., Secatto, A., Belanger, C., Sorgi, C.A., Borgeat, P., Marleau, S., Faccioli, L.H., 2015. Impairment of neutrophil migration to remote inflammatory site during lung histoplasmosis. BioMed Res. Int. 2015, 409309.

Mijatovic, S., Savic-Radojevic, A., Pljesa-Ercegovac, M., Simic, T., Nicoletti, F., Maksimovic-Ivanic, D., 2020. The double-faced role of nitric oxide and reactive oxygen species in solid tumors. Antioxidants 9 (5).

Nair, V., Singh, S., Gupta, Y.K., 2010. Anti-arthritic and disease modifying activity of Terminalia chebula Retz. in experimental models. J. Pharm. Pharmacol. 62 (12), 1801–1806.

Netea, M.G., Nold-Petry, C.A., Nold, M.F., Joosten, L.A., Opitz, B., van der Meer, J.H., van de Veerdonk, F.L., Ferwerda, G., Heinhuis, B., Devesa, I., Funk, C.J., Mason, R.J., Kullberg, B.J., Rubartelli, A., van der Meer, J.W., Dinarello, C.A., 2009. Differential requirement for the activation of the inflammasome for processing and release of IL-1beta in monocytes and macrophages. Blood 113 (10), 2324–2335.

Pang, Y., Liang, M.T., Gong, Y., Yang, Y., Bu, P.L., Zhang, M., Yao, H.C., 2018. HGF reduces disease severity and inflammation by attenuating the NF-kappaB signaling in a rat model of pulmonary artery hypertension. Inflammation 41 (3), 924–931. https://doi.org/10.1007/s10753-018-0747-1.

Parveen, A., Babbar, R., Agarwal, S., Kotwani, A., Fahim, M., 2011. Mechanistic clues in the cardioprotective effect of Terminalia arjuna bark extract in isoproterenol-induced chronic heart failure in rats. Cardiovasc. Toxicol. 11 (1), 48–57.

Patton, L.M., Saggart, B.S., Ahmed, N.K., Leff, J.A., Repine, J.E., 1995. Interleukin-1 beta-induced neutrophil recruitment and acute lung injury in hamsters. Inflammation 19 (1), 23–29.

Piaserico, S., Sandini, E., Saldan, A., Abate, D., 2014. Effects of TNF-alpha inhibitors on circulating Th17 cells in patients affected by severe psoriasis. Drug Dev. Res. 75 (Suppl. 1), S73–S76.

Ramachandran, S., Rajasekaran, A., Manisenthilkumar, K.T., 2012. Investigation of hypoglycemic, hypolipidemic and antioxidant activities of aqueous extract of Terminalia paniculata bark in diabetic rats. Asian Pac. J. Trop. Biomed. 2 (4), 262–268.

Reddy, D.B., Reddanna, P., 2009. Chebulagic acid (CA) attenuates LPS-induced inflammation by suppressing NF-kappaB and MAPK activation in RAW 264.7 macrophages. Biochem. Biophys. Res. Commun. 381 (1), 112–117.

Ribeiro, R.V., Bieski, I.G.C., Balogun, S.O., Martins, D.T.O., 2017. Ethnobotanical study of medicinal plants used by ribeirinhos in the North Araguaia microregion, Mato Grosso, Brazil. J. Ethnopharmacol. 205, 69–102.

Ribeiro, V.P., Arruda, C., Abd El-Salam, M., Bastos, J.K., 2018. Brazilian medicinal plants with corroborated anti-inflammatory activities: a review. Pharm. Biol. 56 (1), 253–268.

Segawa, R., Mizuno, N., Hatayama, T., Jiangxu, D., Hiratsuka, M., Endo, Y., Hirasawa, N., 2016. Lipopolysaccharide-activated leukocytes enhance thymic stromal lymphopoietin production in a mouse air-pouch-type inflammation model. Inflammation 39 (4), 1527–1537.

Singh, M., Kaur, M., Silakari, O., 2014. Flavones: an important scaffold for medicinal chemistry. Eur. J. Med. Chem. 84, 206–239.

So, T., Ishii, N., 2019. The TNF-TNFR family of Co-signal molecules. Adv. Exp. Med. Biol. 1189, 53–84.

Talwar, S., Nandakumar, K., Nayak, P.G., Bansal, P., Mudgal, J., Mor, V., Rao, C.M., Lobo, R., 2011. Anti-inflammatory activity of Terminalia paniculata bark extract against acute and chronic inflammation in rats. J. Ethnopharmacol. 134 (2), 323–328.

Tewari, D., Sah, A.N., Bawari, S., Nabavi, S.F., Dehpour, A.R., Shirooie, S., Braidy, N., Fiebich, B.L., Vacca, R.A., Nabavi, S.M., 2020. Role of Nitric oxide in neurodegeneration: function, regulation and inhibition. Curr. Neuropharmacol. https://doi.org/10.2174/1570159X18666200429001549.

Tsaouli, G., Barbarulo, A., Vacca, A., Screpanti, I., Felli, M.P., 2020. Molecular mechanisms of notch signaling in lymphoid cell lineages development: NF-kappaB and beyond. Adv. Exp. Med. Biol. 1227, 145–164.

Xie, F., 2016. Broad-spectrum antiviral effect of chebulagic acid and punicalagin on respiratory syncytial virus infection in a BALB/c model. Int. J. Clin. Exp. Pathol. 9 (2), 611–619.

Ye, Y., Shan, Y., Bao, C., Hu, Y., Wang, L., 2018. Ginsenoside Rg1 protects against hind-limb ischemia reperfusion induced lung injury via NF-kappaB/COX-2 signaling pathway. Int. Immunopharm. 60, 96–103.

Yeh, C.B., Hsieh, M.J., Hsieh, Y.S., Chien, M.H., Lin, P.Y., Chiou, H.L., Yang, S.F., 2012. Terminalia catappa exerts antimetastatic effects on hepatocellular carcinoma through transcriptional inhibition of matrix metalloproteinase-9 by modulating NF-kappaB and AP-1 activity. Evid. based Compl. Alternat. Med. 595292, 2012.

Zoccal, K.F., Sorgi, C.A., Hori, J.I., Paula-Silva, F.W., Arantes, E.C., Serezani, C.H., Zamboni, D.S., Faccioli, L.H., 2016. Opposing roles of LTB4 and PGE2 in regulating the inflammasome-dependent scorpion venom-induced mortality. Nat. Commun. 7, 10760.