Original Article

A valepotriate-enriched fraction from *Valeriana glechomifolia* Meyer inhibits leukocytes migration and nociception in formalin test in rodents

Tielle M. de Almeida [a], Letícia J. Danielli [b], Miriam A. Apel [b], Eduardo Cassel [c], Rubem M.F. Vargas [c], Gilsane L. Von Poser [b], Liz G. Müller [b], Stela M.K. Rates [a,b,*]

[a] *Programa de Pós-graduação em Ciências Biológicas: Farmacologia e Terapêutica, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil*
[b] *Programa de Pós-graduação em Ciências Farmacêuticas, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil*
[c] *Escola de Engenharia, Departamento de Engenharia Química, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brazil*

ABSTRACT

Previous pre-clinical studies demonstrated that a valepotriates enriched fraction from *Valeriana glechomifolia* F.G. Mey., Caprifoliaceae, was effective against lipopolysaccharide from *Escherichia coli* (LPS)-induced sickness behavior as well as significantly decreased the cortical expression of pro inflammatory cytokines interleukin-1β and tumor necrosis factor-α. Other studies revealed anti-inflammatory properties of *V. wallichii* and *V. amurensis*. These findings open up new perspectives for *Valeriana* genus pharmacology, once it has been commonly associated to sedative and anxiolytic properties. The aim of this study was to investigate the antichemotactic, antinociptive and anti-inflammatory activities of a valepotriate-enriched fraction obtained from aerial and subterranean parts of *V. glechomifolia* submitted to supercritical CO_2 extraction. The biological activities were assessed by means of formalin test in CF1 mice and Wistar rat's leukocytes migration assay (modified Boyden chamber method). Valepotriate-enriched fraction (1, 10 and 30 mg/kg, *p.o.*) inhibited the nociceptive behavior in the late phase of the formalin test in a dose dependent manner. The effect of the valepotriate-enriched fraction highest dose was comparable with that of diclofenac 50 mg/kg (*p.o.*). Valepotriate-enriched fraction (0.1–1 µg/ml) inhibited the leukocyte migration induced by lipopolysaccharide from *Escherichia coli* in a concentration dependent manner. This antichemotatic effect was comparable with that of indomethacin (0.1–1 µg/ml) and better than diclofenac (1 µg/ml) effect. This study demonstrated for the first time that a valepotriate-enriched fraction obtained from *V. glechomifolia* display a peripheral anti-inflammatory like activity.

Introduction

The *Valeriana* genus, Caprifoliaceae, comprises approximately 400 species worldwide distributed (Backlund and Moritz, 1998). Several species from this genus are recognized by their mild sedative, antispasmodic and relaxing properties, and constitute the most popular herbal remedies for treating anxiety and insomnia (Backlund and Moritz, 1998; Hattesohl et al., 2008). Pharmaceutical companies employ *V. officinalis*, *V. wallichii* and *V. edulis* as raw material to produce sedative phytomedicines, which more commonly consist of extracts standardized for valepotriates (Fugg and Cott, 1999; Herrera-Arellano et al., 2001). Additionally, some authors have already shown the antidepressant potential of some *Valeriana* species (Hattesohl et al., 2008; Müller et al., 2012b; 2015b). In this sense, our research group has been studying the pharmacological properties of *Valeriana glechomifolia* F.G. Mey., a species endemic to southern Brazil, which is the one that presents the highest valepotriates content among the Southern Brazil native species (Silva et al., 2002).

The pharmacological properties of plants from *Valeriana* genus are assigned to different constituents, including the monoterpene valerenic acid (Benke et al., 2009), flavonoids (Marder et al., 2003), and valepotriates (Backlund and Moritz, 1998), which comprise a family of terpenes (Geu-Flores et al., 2012) that is only found in the *Valeriana* species (Müller et al., 2015b). The valepotriates frequently found in *Valeriana* species are valtrate, isovaltrate, diavaltrate, acevaltrate, 1-β-acevaltrate and dihydrovaltrate (Bach et al., 1993).

Pre-clinical studies demonstrated antidepressant-like effects of *Valeriana* extracts, which seems to be due to the activation of noradrenergic and dopaminergic neurotransmission at least in part (Hattesohl et al., 2008; Müller et al., 2012b). In line with this assumption, a valepotriates enriched fraction from *V. glechomifolia* displayed a synergistic interaction with classical antidepressants (imipramine, desipramine and bupropion), assessed by isobolographic analysis (Müller et al., 2015c). Moreover, the valepotriate-enriched fraction from *V. glechomifolia* increased Na^+/K^+-ATPase activity in the cortex of mice, as well as the cortical protein expression of the $\alpha 2$ Na^+/K^+-ATPase isoform, which may contribute to its antidepressant-like effect, since decreased Na^+/K^+-ATPase activity has been associated with depression (Müller et al., 2012b). Furthermore, some studies revealed anti-inflammatory properties of *V. wallichii* (Khuda et al., 2013) and *V. amurensis* (Zhang et al., 2010). In addition, pre-clinical studies have shown that *V. officinalis* and *V. glechomifolia* are able to prevent the sickness and depressive-like behavior induced by lipopolysaccharide from *Escherichia coli* (LPS) in rodents, which has been related to neuroinflammation (Neamati et al., 2014; Müller et al., 2015b). Altogether, these findings open up new perspectives for *Valeriana* genus pharmacology.

Considering the above mentioned data, the aim of the present study was to investigate the peripheral anti-inflammatory activity of a valepotriate-enriched fraction (VAL) obtained from the aerial and subterranean parts of *V. glechomifolia* submitted to supercritical CO_2 extraction, by using the formalin test in mice and the Wistar rat's leukocyte migration assay (neutrophil chemotaxis induced by LPS).

Materials and methods

Plant material

Valeriana glechomifolia F.G. Mey., Caprifoliaceae, aerial and subterraneous parts were collected from Southern Brazil. The plant collection was authorized by IBAMA (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis – 29495-1) and accredited by CGEN (Conselho de Gestão do Patrimônio Genético) (A6DABA5). The identification was performed by Dr. M. Sobral (Universidade Federal de São João del-Rei, Minas Gerais, Brazil) and a voucher specimen (Sobral, 7733) was deposited in the Herbarium of the Universidade Federal do Rio Grande do Sul (ICN), Brazil.

Valepotriates fraction preparation

The VAL fraction was obtained according to Müller et al. (2012a). Briefly, 100 g (dry weight) of powdered plant material (aerial and subterraneous parts) were submitted to supercritical CO_2 ($SCCO_2$) extraction using a Pilot Equipment as described by Cassel et al. (2010). The conditions of the extraction were $40\,^\circ C$, 90 bar, $SCCO_2$ flow rate through the extraction vessel: 6.67×10^{-4} kg/s. The $SCCO_2$ extraction yield was 3.23%.

Valepotriates fraction characterization

The VAL fraction was dissolved in HPLC grade methanol and filtered ($0.22\,\mu m$ pore size, Merck$^©$) before the analysis by HPLC according to a method previously described (Silva et al., 2002; Müller et al., 2012a), using Shimadzu HPLC system and Waters Nova-Pack C18 column ($4\,\mu m$, 3.9×150 mm i.d. with Waters Nova-Pack C-18 guard column, 60° A, 3.9×20 mm). The isocratic mobile phase (acetonitrile:water (50:50 v/v)); at 1 ml/min; UV detection at 254 nm. The samples were dissolved in HPLC grade methanol and filtered through a membrane filter (0.22 mm pore size, Merck$^©$).

Valtrate was used as internal standard. The compound was isolated from the fraction, purified by column chromatography, using the same procedure described by Salles et al. (2000) and identified by ^1H and ^{13}C NMR. The other valepotriates have already been characterized by ^1H and ^{13}C NMR by our research group (Salles, 1999; Salles et al., 2000). Since diene valepotriates are structurally similar and present the same chromophore group, it is possible to express their concentration in terms of a single product. For achievement of the calibration curve, valtrate was diluted (in grade HPLC methanol) stepwise (250; 125; 62.5; 31.25; 15.62; 7.81 mg/ml) and $20\,\mu l$ were injected into the HPLC in triplicate. Linearity analysis of the calibration curve revealed $R^2 = 0.9999$. The valepotriates fraction was diluted (250 mg/ml) and $20\,\mu l$ were injected into the HPLC in triplicate. The compounds were identified by using their retention time. The retention time of the valepotriates were: valtrate 26.5 min, isolvatrate 22 min, acevaltrate 14 min, 1-β-acevaltrate 11.5 min and for 1-β-aceacevaltrate 8.5 min. All valepotriates were quantified in mg of valtrate equivalent/g fraction.

Animals

Experiments were carried out using male CF1 mice (30–45 g) and male Wistar rats (180–220 g) from Centro de Reprodução e Experimentação de Animais de Laboratório – CREAL – UFRGS, Rio Grande do Sul, Brazil. Animals were housed in plastic cages at $23 \pm 1\,^\circ C$ under a 12-h light/dark cycle, with food and water provided *ad libitum*. Experiments were approved by Animal Care Local Ethical Committee (CEUA-UFRGS; 28603, April 16th, 2015) and were conducted in accordance with Brazilian law (Portaria no. 596, de 25 de junho de 2013, Portaria no. 465, de 23 de maio de 2013 and Lei no. 11.794, de 8 de outubro de 2008) and European Communities Council Directive of 24 November 1986 (86/609/EEC).

Formalin test

Different groups of animals (6–10 mice/group) were treated with VAL (1, 10 and 30 mg/kg), diclofenac 50 mg/kg (positive control) or saline (negative control) 1 h before the formalin injection. The doses range followed previous studies by our group (Müller et al., 2012b). The animals were adapted to the apparatus (an acrylic squad chamber 20 cm wide × 30 cm high) for 20 min before receiving an intra-plantar (*i.pl.*) injection of formalin. Immediately, the animals were observed during the first 5 min (neurogenic phase) and between the 15th and 30th min (inflammatory phase). The time that animal spent licking and biting the injected paw (during the first and second phases of the test) was recorded with a chronometer and was considered an indicative of nociceptive behavior (Basting et al., 2014; Holanda et al., 2015).

VAL was suspended in saline (0.1, 1 and 3 mg/ml) with addition of 1% (v/v) polysorbate 80 and sonicated for 1 min prior the administration. All treatments were administered by gavage at 10 ml/kg body weight. Twenty microliters of 2% formalin (formaldehyde) diluted in saline were injected subcutaneously into the plantar surface (*i.pl.*) of the right hind paw of mice.

Antichemotactic assay *in vitro*

Experiments were carried out according to the modified Boyden chamber method (Suyenaga et al., 2011). A total of seven animals were used in this assay. For obtaining rat polymorphonuclear neutrophils, 20 ml of sterile 1% glycogen (w/v) was injected into the peritoneum of one Wistar rat that 4 h later was killed for leukocytes collection. Prior to the chemotaxis assay, neutrophils were treated with VAL and indomethacin in concentrations of 0.1 to 1 µg/ml or diclofenac (1 µg/ml) at $37\,^\circ C$ for 30 min. For plasma obtention, six rats were used. The plasma was incubated at $37\,^\circ C$ for 30 min

with 65 μg/ml of LPS (lipopolysaccharide from *E. coli*) and diluted in Hanks buffer to a 20% solution (v/v). The leukocyte/samples were added in the upper wells of the chamber, separated by an 8 μm nitrocellulose filter (Millipore, USA) from the chemotactic stimulant (Lipopolysaccharide from *E. coli* – LPS) present in the bottom compartment. Then, the chamber was kept at 37 °C for 1 h. The leucocytes migration through the filter was measured by using an optical microscope. The distance from the top of the filter to the farthest plane of focus containing two cells, in five microscopic fields of duplicate filters allowed the evaluation of leukocyte migration.

VAL stock solution (1 mg/ml) was prepared by using Hanks' balanced salt solution (HBSS) with addition of 1% (v/v) polysorbate 80 and sonicated for 1 min. The reference drugs indomethacin and diclofenac were also dissolved in HBSS. The concentration of polysorbate 80 in all final working solutions was less than 0.01%. The HBSS plus polysorbate 80 was used as negative control.

Statistical analysis

The results from neurogenic phase of formalin test and from antichemotactic assay were analyzed by ANOVA followed by Tukey's test. Data from inflammatory phase were analyzed by Kruskal–Wallis test followed by Dunn's multiple comparison test because these data were not normally distributed; $p < 0.05$ was considered statistically significant. Data analyses were performed using the GraphPad Prism 7.0 software.

Results and discussion

The present study demonstrated for the first time that a valepotriate-enriched fraction (VAL) from aerial and subterraneous parts (the whole plant) of *V. glechomifolia* obtained by supercritical CO_2 extraction was effective in the second phase of the mice formalin test and displayed antichemotactic activity in the leukocyte migration assay. These data suggest an anti-inflammatory effect of valepotriates.

The characterization of the VAL fraction, performed by HPLC, indicated that valepotriates seem to be its main constituents. Among them, valtrate takes place in largest quantity, followed by acevaltrate, 1β-acevaltrate, 1β-aceacevaltrate, and isovaltrate (Fig. 1). These compounds have been previously isolated from *V. glechomifolia* by our research group (Salles et al., 2000; Cassel et al., 2010). Table 1 depicts the concentration of each main valepotriate.

Fig. 2(A) shows the effects of VAL in the nociceptive (first) phase of the formalin test. The One way ANOVA [$F_{(4, 25)} = 0.772$, $p = 0.5537$] did not reveal any significant difference between the nociceptive behavior time of all groups. VAL produced

Table 1
Valeptriates content (mg/g supercritical CO_2 *Valeriana glechomifolia* fraction: VAL) determined through HPLC.

Valepotriate	mg of valtrate equivalent/g of VAL
Valtrate	230 ± 4.04
Acevaltrate	193 ± 4.06
1-β-Acevaltrate	58 ± 0.87
1-β-Aceacevaltrate	14 ± 0.98
Isovaltrate	13 ± 0.95

The values are expressed as mean ± SD.

antinociceptive effect only in the second phase of the formalin test (Fig. 2(B)), suggesting that it presents an activity similar to anti-inflammatory drugs (*e.g.*, steroidal or non-steroidal anti-inflammatory drugs) (Holanda et al., 2015) which suppress only the second phase of formalin test. Kruskal–Wallis analysis revealed that the groups treated with diclofenac (positive control) and VAL 30 mg/kg feature a time of nociceptive behavior significantly lower that the group treated with saline [$H = 10.11$; $p = 0.0386$]. There was no significant difference between the nociceptive behavior of groups treated with diclofenac and VAL 30 mg/kg ($p = 0.1171$). It is also possible to observe that the effect of VAL 30 mg/kg was significantly higher than VAL 1 and 10 mg/kg ($p < 0.05$), which indicates a dose dependent effect.

It is well established that the formalin produces a biphasic behavioral reaction with an initial phase within the first (0–5) min post injection, followed by a quiescent period (approximately 15 min), and a second phase of nociceptive behaviors lasting 15–30 min. The first phase relates to the direct stimulation of nociceptors and is sensitive to local anesthetics and opiates, while the second phase corresponds to inflammatory responses and central sensitization within the dorsal horn (Coderre et al., 2013). The second phase responds to various drugs with recognized analgesic and/or anti-inflammatory efficacy, such as steroidal or non-steroidal anti-inflammatory drugs, *N*-methyl-D-aspartate antagonists and gabapentin (Holanda et al., 2015).

These results are in line with other studies on the anti-inflammatory effects of species of *Valeriana*. Khuda et al. (2013) demonstrated that a topical formulation of crude methanol extract of *V. wallichii* showed anti-inflammatory effect in the carrageenan induced hind paw edema model in rats. These authors suggested that a possible mechanism of the anti-inflammatory activity of *V. wallichii* is the inhibition of histamine, serotonin and prostaglandins synthesis. Another study demonstrated that an ethanolic extract of *V. amurensis* controlled the inflammatory reaction in the cortex and hippocampus of rats submitted to a model
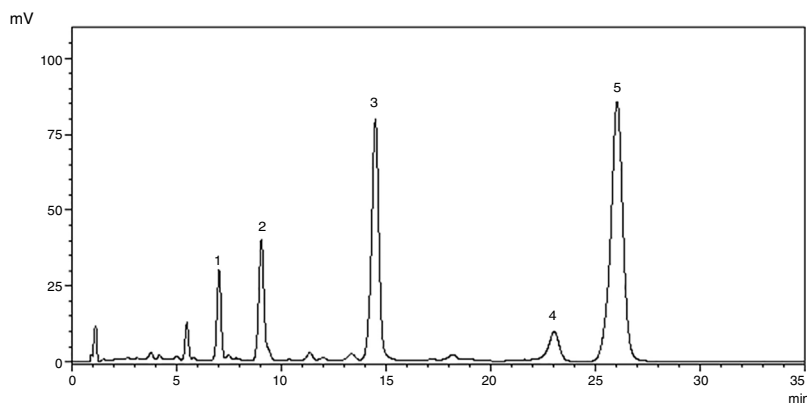
Fig. 1. Representative HPLC chromatogram of the *Valeriana glechomifolia* (VAL) supercritical carbon dioxide extract. Compounds present are: (1) 1-β-aceacevaltrate, (2) 1-β-acevaltrate, (3) acevaltrate, (4) isovaltrate and (5) valtrate.
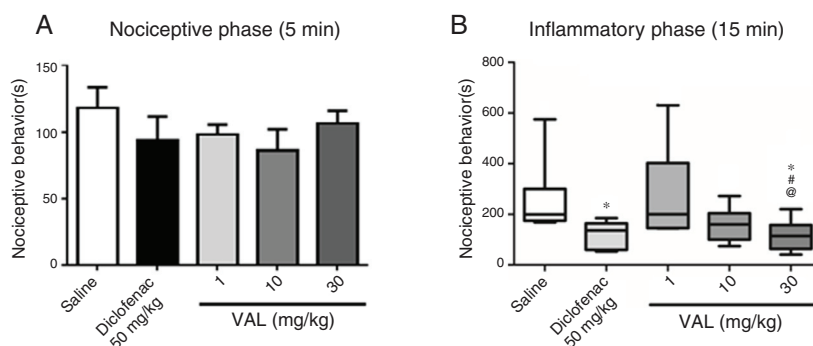
Fig. 2. Effect of a valepotriates fraction from _Valeriana glechomifoliana_ aerial and subterraneous parts (VAL) in the nociceptive (A) and inflammatory (B) phases of the behavioral test of formalin. Mice were treated (p.o.) with VAL 1 h before the formalin injection (i.pl.) (2%, 20μl). The nociceptive behavior time was recorded for the first 5 min after the formalin injection (i.pl.). One-way ANOVA (n = 6-10 mice / group). Data are expressed as mean ± S.E.M. The nociceptive behavior was recorded during the final 15 min (from a 30 min session) after the injection of formalin. Kruskal-Wallis test followed by Dunn's multiple comparison test, * different from saline-treated group, P < 0.05 ; # different from VAL 1 mg/kg, P < 0.05 ; @ different from VAL 10 mg/kg, P< 0.05 . The box plots represent the median and quartiles 25% and 75% as the lower and upper edges of the boxes, respectively (n = 6-10 mice / group).
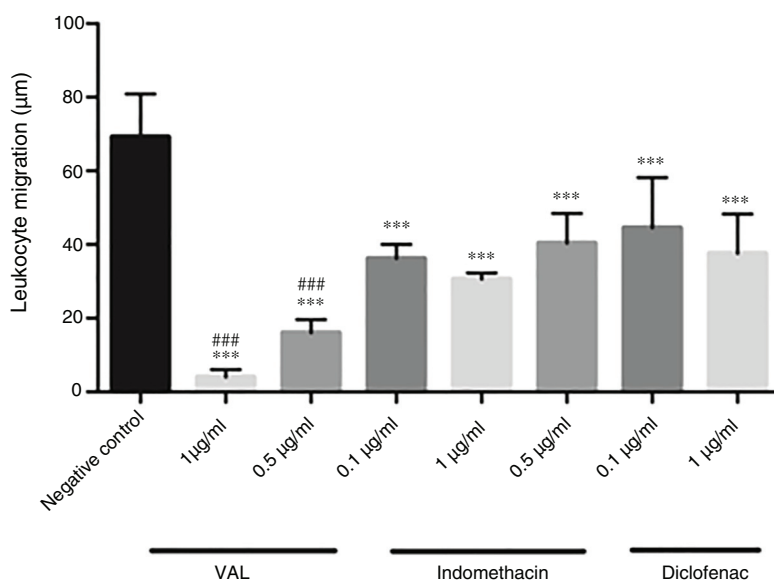
Fig. 3. Effect of a valepotriates fraction from _Valeriana glechomifolia_ aerial and subterraneous parts (VAL) on the polymorphonuclear neutrophil chemotaxis _in vitro_. The leukocytes were treated with a range of 0.1–1.0 μg/ml of VAL at 37 °C for 1 h. Chemotaxisis expressed as mean ± SEM of leukocyte migration. ***$p < 0.0001$ indicates a significant difference compared to negative control; ###$p < 0.001$ indicates a significant difference when compared to VAL 0.1 μg/ml and all concentrations of indomethacin and diclofenac.

of Alzheimer's disease (Zhang et al., 2010). The mechanism of anti-inflammatory action from _V. amurens_ ethanolic extract appears to be the reduction of iNOS, COX-2 and ikappaB-alpha expression in cortical and hippocampal neurons (Zhang et al., 2010). Dong et al. (2018) demonstrated that valepotriates from _V. jatamansi_ are able to inhibit _N_-type calcium channels. This pharmacological property is probably related to the analgesic effect of _V. jatamansi_. These findings are particularly relevant, and we may speculate that the effectiveness of the valepotriates-enriched fraction from _V. glechomifolia_ in attenuating nociception in the formalin test might be related to the blockage of _N_-type calcium channels. However, this hypothesis deserves further investigation. _Valeriana officinalis_ prevented the development of sickness behavior and depressive-like behavior induced by LPS in rodents (Neamati et al., 2014). Müller et al. (2015a) demonstrated that VAL was also effective against LPS-induced sickness behavior as well as significantly decreased the cortical expression of pro-inflammatory cytokines IL-1β and TNF-α.

During the acute phase of inflammation, there is a release of pro-inflammatory mediators including bioactive amines, lipid mediators and cytokines, typically TNF-α and IL-1β, which produces a chemotactic gradient to guide and activate recruited cells to the site of injury (Coutinho and Chapman, 2011). Cytokines constitute a link between cellular injuries or immunological recognition and the local or systemic signs of inflammation, _e.g._ cell migration, edema, fever, and hyperalgesia (Dinarello, 2000).

Considering that the suppression of neutrophil functions control inflammatory responses and it is part of the mechanism of action of some non-steroidal anti-inflammatory (Sugimoto et al., 2016), the effects of VAL on leukocyte chemotaxis induced by LPS were investigated in the Boyden chamber _in vitro_ method. Bacterial LPS has been extensively used in models studying inflammation as it mimics many inflammatory effects of cytokines, such as TNF-α, IL-1β or IL-6. This activity seems to be mediated by small G proteins facilitating the release of pro-inflammatory cytokines (Ngkelo et al., 2012).

The results of chemotaxis assay are shown in Fig. 3. One-way ANOVA [$F(7, 49) = 41.93$; $p < 0.001$] revealed that VAL significantly inhibited leukocyte migration at all concentrations tested when compared to negative control ($p < 0.001$). VAL and

indomethacin displayed a concentration dependent response. Diclofenac was tested at a unique concentration because this drug is effective only at high concentrations in this assay (Pakauskas et al., 2011). VAL 1 µg/ml and 0.5 µg/ml significantly inhibited leukocyte migration when compared to VAL 0.1 µg/ml and all concentrations of indomethacin and diclofenac ($p < 0.001$). These results demonstrate that VAL presents a better effect than the reference drugs used on the inhibition of neutrophil migration. In line with these results, other studies also showed the antichemotatic activity of other terpenoids, such as 1,8-cineole (Ahumada et al., 1997), 28,28,30-trihydroxylupeol, 3,21,21,26-tetrahydroxylanostanoic acid, dehydroxybetulinic acid, taraxerone, ethyl palmitate and ursolic acid (Mawa et al., 2016). Notably, it has been shown that the systemic administration of the terpene rose-oxide inhibited key events related to inflammation, namely edema, local increase of IL-1β level, and leukocyte migration, producing consistent anti-inflammatory effects in different models of inflammation in mice and rats, including formalin test and leukocyte migration (Nonato et al., 2012).

Conclusion

In conclusion, the results so far demonstrated for the first time that that a valepotriate-enriched fraction obtained from *V. glechomifolia* displays biological effects that indicate a peripheral anti-inflammatory activity corroborating with other studies showing the anti-inflammatory activity of *Valeriana* species. The anti-inflammatory like activity might be at least in part related to its ability to inhibit the expression of pro-inflammatory cytokines (IL-1β and TNF-α), previously reported by Müller et al. (2015a), as well as to its capacity for inducing leukocyte migration. These results highlight the need for further investigations of valepotriates, which are a special class of terpenes occurring in *Valeriana*, as potential prototypes for the development of new drugs to treat inflammatory conditions.

Ethical Disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Authors' contribution

Study concept and design: TMA, LJD, MAA, LGM, SMKR. Acquisition of data: TMA, LJD, EC, RMFV. Analysis and interpretation of data: TMA, LJD, MAA, EC, RMFV, GLVP, LGM, SMKR. Drafting of the manuscript: TMA, LJD, LGM, MAA, SMKR. Study supervision: LGM, SMKR. All authors have read and approved the final version of the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

References

Ahumada, C., Sáenz, T., García, D., Puerta, R.L., Fernandez, A., Martinez, E., 1997. The effects of a triterpene fraction isolated from *Crataegus nonogyna* Jacq. on different acute inflammation models in rats and mice. Leucocyte migration and phospholipase A_2 inhibition. J. Pharm. Pharmacol. 49, 329–331.

Bach, K.K., Ghia, F., Torssell, K.B., 1993. Valtrates and lignans in *Valeriana microphylla*. Planta Med. 59, 478–479.

Backlund, A., Moritz, T., 1998. Phylogenetic implications of an expanded valepotriate distribution in the Valerianaceae. Biochem. Syst. Ecol. 26, 309–335.

Basting, R.T., Nishijima, C.M., Lopes, J.A., Santos, R.C., Lucena Périco, L., Laufer, S., Bauer, S., Costa, M.F., Santos, L.C., Rocha, L.R., Vilegas, W., Santos, A.R., Dos Santos, C., Hiruma-Lima, C.A., 2014. Antinociceptive, anti-inflammatory and gastroprotective effects of a hydroalcoholic extract from the leaves of *Eugenia punicifolia* (Kunth) DC. in rodents. J. Ethnopharmacol. 157, 257–267.

Benke, D., Barberis, A., Kopp, S., Altmann, K.H., Schubiger, M., Vogt, K.E., Rudolph, U., Möhler, H., 2009. $GABA_A$ receptors as in vivo substrate for the anxiolytic action of valerenic acid, a major constituent of valerian root extracts. Neuropharmacology 56, 174–1817.

Cassel, E., Vargas, R.M.F., Brun, G.W., Almeida, D.E., Cogoi, L., Ferraro, G., Filip, R., 2010. Supercritical fluid extraction of alkaloids from *Ilex paraguariensis* St. Hil. J. Food Eng. 100, 656–661.

Coderre, T.J., Abbott, F.V., Sawynok, J., 2013. Encyclopedia of Pain: Formalin Test. Springer Berlin Heidelberg.

Coutinho, A.E., Chapman, K.E., 2011. The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. Mol. Cell. Endocrinol. 335, 111–222.

Dinarello, C.A., 2000. Proinflammatory cytokines. Chest 118, 503–508.

Dong, F.W., Jiang, H.H., Yang, L., Gong, Y., Zi, C.T., Yang, D., Ye, C.J., Li, H., Yang, J., Nian, Y., Zhou, J., Hu, J.M., 2018. Valepotriates from the roots and rhizomes of *Valeriana jatamansi* Jones as novel *N*-type calcium channel antagonists. Front. Pharmacol. 9, 1–9.

Fugg, B.A., Cott, J.M., 1999. Dietary supplements and natural products as psychotherapeutic agents. Psychosom. Med. 61, 712–728.

Geu-Flores, F., Sherden, N.H., Courdavault, V., Burlat, V., Glenn, W.S., Wu, C., Nims, E., Cui, Y., O'Connor, S.E., 2012. An alternative route to cyclic terpenes by reductive cyclization in iridoid biosynthesis. Nature 492, 138–142.

Hattesohl, M., Feistel, B., Sievers, H., Lehnfeld, R., Hegger, M., Winterhoff, H., 2008. Extracts of *Valeriana officinalis* L. show anxiolitic and antidepressant effects but neither sedative nor myorelaxant properties. Phytomedicine 15, 2–15.

Herrera-Arellano, A., Luna-Villegas, G., Cuevas-Uriostegui, M.L., Alvarez, L., Vargas-Pineda, G., Zamilpa-Alvarez, A., Tortoriello, J., 2001. Polysomnographic evaluation of the hypnotic effect of *Valeriana edulis* standardized extract in patients suffering from insomnia. Planta Med. 67, 695–699.

Holanda, V., Asth, L., Santos, A.R., Guerrini, R., Soares-Rachetti, V.P., Calo, G., André, E., Gavioli, E.C., 2015. Central adenosine A_1 and A_{2A} receptors mediate the antinociceptive effects of neuropeptide S in the mouse formalin test. Life Sci. 120, 8–12.

Khuda, F., Iqbal, Z., Khan, A., Nasir, F., Shah, Y., 2013. Anti-inflammatory activity of the topical preparation of *Valeriana wallichii* and *Achyranthes aspera* leaves. Pak. J. Pharm. Sci. 26, 451–454.

Marder, M., Viola, H., Wasowski, C., Fernández, S., Medina, J.H., Paladini, A.C., 2003. 6-Methyl apigenin and hesperidin: new Valeriana flavonoids with activity on the CNS. Pharmacol. Biochem. Behav. 75, 537–545.

Mawa, S., Jantan, I., Husain, K., 2016. Isolation of terpenoids from the stem of *Ficus aurantiaca* griff and their effects on reactive oxygen species production and chemotactic activity of neutrophils. Molecules 21, http://dx.doi.org/10.3390/molecules21010009.

Müller, L.G., Borsoi, M., Stolz, E.D., Herzfeldt, V., Viana, A.F., Ravazzolo, A.P., Rates, S.M.K., 2015a. Diene valepotriates from *Valeriana glechomifolia* prevent lipopolysaccharide-induced sickness and depressive-like behavior in mice. Evid.-Based Complement. Altern. Med., http://dx.doi.org/10.1155/2015/145914.

Müller, L.G., de Andrade Salles, L., Sakamoto, S., Stein, A.C., Cargnin, S.T., Cassel, E., Vargas, R.F., Rates, S.M., von Poser, G.L., 2012a. Effect of storage time and conditions on the diene valepotriates content of the extract of *Valeriana glechomifolia* obtained by supercritical carbon dioxide. Phytochem. Anal. 23, 222–227.

Müller, L.G., Salles, L., Lins, H.A., Feijó, P.R., Cassel, E., Vargas, R., von Poser, G.L., Noël, F., Quintas, L.E., Rates, S.M., 2015b. Effects of diene valepotriates from *Valeriana glechomifolia* on Na^+/K^+-ATPase activity in the cortex and hippocampus of mice. Planta Med. 81, 200–207.

Müller, L.G., Salles, L.A., Stein, A.C., Betti, A.H., Sakamoto, S., Cassel, E., Vargas, R.F., von Poser, G.L., Rates, S.M., 2012b. Antidepressant like effect of *Valeriana gle-*

chomifolia Meyer (Valerianaceae) in mice. Prog. Neuropsychopharmacol. Biol. Psychiatry 36, 101–109.

Müller, L.G., Stolz, E.D., Betti, A.H., Herzfeldt, V., Rates, S.M., 2015c. Synergistic interaction between diene valepotriates from Valeriana glechomifolia Meyer. J. Pharm. Pharmacol. 67, 1008–1016.

Neamati, A., Chaman, F., Hosseini, M., Boskabady, M.H., 2014. The effects of Valeriana officinalis L. hydro-alcoholic extract on depression like behavior in ovalbumin sensitized rats. J. Pharm. Bioallied. Sci. 6, 97–103.

Ngkelo, A., Meja, K., Yeadon, M., Adcock, I., Kirkham, P.A., 2012. LPS induced inflammatory responses in human peripheral blood mononuclear cells is mediated through NOX4 and $G_i\alpha$ dependent PI-3kinase signaling. J. Inflamm. 9, http://dx.doi.org/10.1186/1476-9255-9-1.

Nonato, F.R., Santana, D.G., Melo, F.M., Santos, G.G.L., Brustolim, D., Camargo, E.A., de Sousa, D.P., Soares, M.B., Villarreal, C.F., 2012. Anti-inflammatory properties of rose oxide. Int. Immunopharmacol. 14, 779–784.

Pakauskas, S., Parseliunas, A., Kerkadze, V., Nobiling, R., Schmidt, J., Ryschich, E., 2011. Blockade of leukocyte haptokinesis and haptotaxis by ketoprofen, diclofenac and SC-560. BMC Immunol. 64, http://dx.doi.org/10.1186/1471-2172-12-64.

Salles, L.A., M.Sc. Thesis 1999. Estudo fitoquímico e estabelecimento de cultura de calos de Valeriana glechomifolia Meyer. Pharmacy School, Federal University of Rio Grande do Sul.

Salles, L.A., Silva, A., Rech, S.B., Zanatta, N., von Poser, G.L., 2000. Constituents of Valeriana glechomifolia Meyer. Biochem. Syst. Ecol. 28, 907–910.

Silva, A.L., Rech, S.B., von Poser, G.L., 2002. Quantitative determination of valepotriates from Valeriana native to south Brazil. Planta Med. 68, 570–572.

Sugimoto, M.A., Sousa, L.P., Pinho, V., Perretti, M., Teixeira, M.M., 2016. Resolution of inflammation: what controls its onset? Front. Immunol. 7, http://dx.doi.org/10.3389/fimmu.2016.00160.

Suyenaga, E.S., Konrath, E.L., Dresch, R.R., Apel, M.A., Zuanazzi, J.A., Chaves, C., Henriques, A.T., 2011. Appraisal of the antichemotactic activity of flavonoids on polymorphonuclear neutrophils. Planta Med. 77, 698–704.

Zhang, Z.L., Zuo, Y.M., Wang, Q.H., Xiao, H.B., Kuang, H.X., 2010. Effects of Valeriana amurensis on the expressions of iNOS, COX-2 and IkappaCB-alpha in Alzheimer's disease model rat's brain. Zhong Yao Cai 33, 581–583.