

Anti-inflammatory activity of a natural herbal-marine drug (MS₁₄ - SANT and SUSP) compared to sodium salicylate or methylprednisolone in a rat model for multiple sclerosis

Mohsen Naseri (1,2), Hossein Rezaeizadeh (3), Sayid Mahdi Mirghazanfari (4), Taiebeh Taheripana (5), Zahra Bararpour (6), Neda Ashayeri (7), Majid Asghari (8), Abbas Talebi Mazrae Shahi (1), Fatemeh Emadi (1,2), Parvaneh Mohseni Moghaddam (9), Abbas Hasheminejad (2), Farzaneh Ghaffari (10)

(1) Traditional Medicine Clinical Trial Research Center, Shahed University, Tehran, Iran; (2) Department of Traditional Persian Medicine, Faculty of Medicine, Shahed University, Tehran, Iran; (3) Department of Persian Medicine, School of Persian Medicine, Tehran University of Medical Sciences, Tehran, Iran; (4) Department of Physiology and Iranian Medicine, School of Medicine, AJA University of Medical Sciences, Tehran, Iran; (5) Besat Hospital, AJA University of Medical Sciences, Tehran, Iran; (6) Department of Microbiology, Faculty of Medicine, Shahed University, Tehran, Iran; (7) Pediatric Hematology and Oncology Department, Ali Asghar children hospital, Iran University of Medical Sciences, Tehran, Iran; (8) Traditional Medicine Research Center, School of Traditional Medicine, Qom University of Medical Science, Qom, Iran; (9) Department of Physiology, Faculty of Medicine, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran; (10) School of Traditional Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

This article is distributed under the terms of the Creative Commons Attribution Noncommercial License (CC BY-NC 4.0) which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

Abstract

A natural compound of marine herbal origin has been used in Persian Traditional Medicine to relieve some symptoms of multiple sclerosis. The present study investigated the anti-inflammatory effects of a patented extracts of the traditional receipt (MS₁₄). In this preliminary experiment, we used seven groups of six rats: the control group received vehicle, the two positive control groups were treated with either sodium salicylate (300 mg/kg) intraperitoneal (i.p.) or methyl prednisolon (MPN 10 mg/kg) i.p., while the test groups were treated with a solution centrifuged MS₁₄ (SANT 100 mg/kg) and suspension of MS₁₄ (SUSP 100, 150, 300 mg/kg) i.p. After thirty minutes, paw volume was measured by plethysmometer and immediately formalin solution was injected subcutaneously into the hind paw and after an hour, inflamed paw volume was measured. In days 2-8, the inflamed paw volume was measured and immediately drugs were injected i.p. The anti-inflammatory effect of MPN was significant only on days 5 and 6. The anti-inflammatory effect of SS was significant only on the 6th day, while the anti-inflammatory effect of SANT MS₁₄ (100 mg/kg) was also significant only on the 6th day. SUSP MS₁₄ (150 mg/kg) significantly reduced edema from second to 6th day. Intra-peritoneal injection of SUSP MS₁₄ with 300 mg/kg was toxic, so excluded from the study. This research indicates that the MS₁₄ possesses an anti-inflammatory effect after intra-peritoneal administration. Comparative anti-inflammatory effects of MS₁₄ with Glucocorticoids in this study, may justify a possible mechanism for its action in multiple sclerosis, if further studies will provide strong statistically confirmatory effects in animals and safety human trials.

Key Words: Traditional Persian Medicine; *Perna latissculatus*; *Apium graveolens*; *Hypericum perforatum*; MS₁₄; anti-inflammatory action; *in vivo* animal experiments.

Eur J Transl Myol 32 (1): 10169, 2022 doi: 10.4081/ejtm.2022.10169

Multiple sclerosis (MS) is the most common cause of neurological disability in young adults and is

characterized by inflammation, demyelination and gliosis in the central nervous system.¹ More recently the

widespread involvement of grey matter, particularly early cortical lesions, has received much attention.² Inflammation has been stated to have a central role in the pathophysiology of multiple sclerosis. However, genetic data, imaging studies, and immunopathological findings challenge this view.³ Current available drugs for MS are highly toxic and are non-selective and so development of a less toxic, integrative therapies for its control is necessary.⁴⁻⁷ One of the most potent anti-inflammatory and immunosuppressive available drugs are Glucocorticoids but the use of these drugs is not without side effects.¹

Traditional Persian Medicine (TPM) consists of huge practical experiences for disease prevention and treatment that have been used from more than 10,000 years.^{8,9} It could be considered as a potential source for finding novel treatment strategies as shown in some recent researches.^{10,11} In TPM, the emphasis is more on prevention rather than treatment. In TPM, life style modification, specially nutrition,^{12,13} is at the core of treatment and prevention of diseases, while pharmacotherapy and manipulation are the second and third approach.¹⁴ TPM may provide solutions to solve difficult problems in the field of neurological and psychological diseases, such as headache, chronic sciatica, spinal cord trauma and depression.¹⁴⁻¹⁷

MS₁₄ is an herbal-marine drug containing *Penaeus latisculatus* (Meygu in Persian), *Apium graveolens* (Karafs in Persian) and *Hypericum perforatum* (Hufarighun in Persian). It has been used in TPM as an anti-inflammatory drug to relieve pain in multiple sclerosis.⁸⁻²⁰ It has been patented at the registration office of Islamic Republic of Iran (no: 29350) and classified as equivalent to food with no observable adverse effect level (NOAEL).²¹ Our previous double blind, placebo-controlled, crossover study have shown that MS₁₄ improve mobility and quality of life of patients.²² Furthermore, in a rodent model study MS₁₄ ameliorated mice Experimental Allergic Encephalomyelitis (EAE) with its anti-inflammatory activity and attenuated inflammation in central nervous system (CNS).²³

Accordingly, to confirm the mechanisms of action of MS₁₄, an experimental model in rats was designed to investigate the anti-inflammatory effects of MS₁₄ in comparison to nonsteroidal anti-inflammatory drugs (NSAIDs) and Glucocorticoids.

Materials and Methods

Drugs

MS₁₄ was supplied by the Traditional Medicine Clinical Trial Research Center, Shahed University (Tehran, Iran). Sodium salicylate, formaldehyde, sodium hydroxide and hydrochloric acid were purchased from E. Merck (Darmstadt, Germany) and methyl prednisolon from Abureihan Company (Tehran, Iran).

The plant materials

MS₁₄ contained 90% *Penaeus latisculatus* (King prawn), 5% *Apium graveolens* L. (Wild celery) and 5% *Hypericum perforatum* L. (St John's wort).

Preparation of MS₁₄

MS₁₄ was dissolved in proportional portions with hydrochloric acid. The mixture was shaken vigorously and neutralized with sodium hydroxide to reach pH 7.0, and then three suspensions with different concentration were produced. Thereafter, another sample was centrifuged and supplied pure solution.²⁴

Animals

Male Albino rats (230-330g) from Physiology Department (Tehran University of Medical Sciences, Iran) were used. The animals were housed in standard cages with free access to food (standard laboratory rodent's chow) and water. The animal house temperature was maintained at 23±3 °C with a 12-h light/dark cycle (light on from 06:00 to 18:00 h). The ethical guidelines for the investigation on animals were followed in all tests. All efforts were made to minimize animal distress and to reduce the number of animals used. In this study, experimental animals were divided randomly into seven groups with six animals in each group (n=6).^{25,26}

Group I: The control group received vehicle, intraperitoneal (i.p.);

Groups II - III: The positive control groups were treated with 300 mg/kg dose of sodium salicylate (SS) or 10 mg/kg methyl prednisolon (MPN), (i.p.);

Groups IV-VII: The test groups were treated with centrifuged MS₁₄ (SANT 100 mg/kg) and suspension of MS₁₄ (SUSP 100, 150, 300 mg/kg) i.p., respectively.

Anti-inflammatory studies

Formalin test: The test was performed in accordance with the method of Dubuisson and Dennis.²⁷

Rats were injected with 0.05 ml of formaldehyde 2.5% (v/v in distilled water) into the subcutaneous region of the left hind paw. Drugs were injected 30 min before formalin injection and the paw volume was measured by using plethysmometer just before and 1h after formalin injection (1.5 h after drug injection). In days 2-8, animals received drugs just after paw volume measurement. The paw inflammation was measured using a modified method.^{28,29}

Statistical analysis

Data are presented as means ± standard deviations. The one-way analysis of variance (ANOVA) followed by the (LSD)'s post-test was used to analyze the data obtained from formalin test. P < 0.05 was the critical criterion for statistical significance. SPSS statistics program version 11.5 was used for data analysis.

Results

Anti-inflammatory activity

Single doses effects of different doses of MS₁₄, of SS and of MPN are shown in Figure 1. The administration of MS₁₄ with doses of 300 mg/kg, significantly inhibited the paw edema ($P < 0.05$ compared with control group).

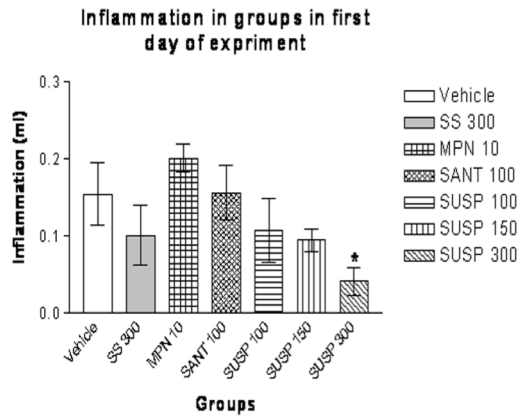


Fig 1. Effect of the MS₁₄ on paw edema induced by formalin
Data represent mean $\pm$ S.E.M. for six rats.
* $P < 0.05$ compared with control group.
SS: sodium salicylate; MPN: methyl prednisolon; SANT: centrifuged MS₁₄; SUSP: suspension of MS₁₄

Results of serial administration of different doses of MS₁₄, SS and MPN are shown in Figure 2 and Figure 3. As is shown in these Figures, anti-inflammatory effect of MPN (10 mg/kg) was significant only in days 5 and 6, and anti-inflammatory effect of SS (300 mg/kg) was significant only in 6th day, anti-inflammatory effect of MS₁₄ (SANT 100 mg/kg) was also significant only in 6th day while MS₁₄ (SUSP150 mg/kg) significantly reduced edema from second to 6th day. Intra-peritoneal injection of MS₁₄ with 300 mg/kg was toxic, so it was excluded from the study.

Discussion

Multiple sclerosis is a chronic inflammatory, autoimmune disease of the CNS.³⁰ As the etiology and triggers factors of MS are poorly understood, its treatment and study is difficult.⁴ Like many other diseases, diet and lifestyle modification, dietary supplementation, and moderate physical activity can improve quality of life in MS, but medical therapies also show promise. Supplementation of the diet with vitamins, minerals as calcium and magnesium, anti-inflammatory diet, flavonoids derived from plant sources and other nutrients is indicated for patients well being and for improvement of deficiencies caused by demyelinating and autoimmune-inflammatory process. Some of the plants applied in traditional medicine may have useful effects in MS₁₄. Thus, we evaluated the putative anti-inflammatory activities of

the MS₁₄ to clarify the mechanism of traditional belief in the relieving symptoms of multiple sclerosis by this compound.

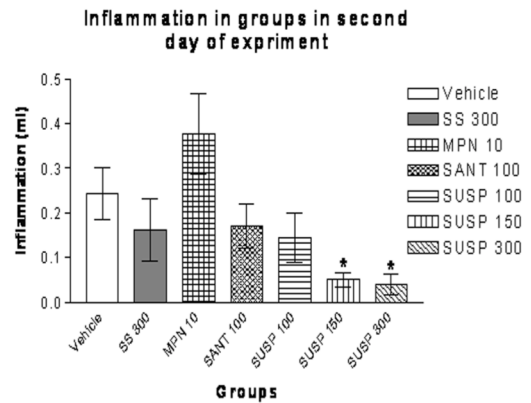


Fig 2. Effect of the MS₁₄ on paw edema induced by formalin
Data represent mean $\pm$ S.E.M. for six rats.
* $P < 0.05$ compared with control group.
SS: sodium salicylate; MPN: methyl prednisolon; SANT: centrifuged MS₁₄; SUSP: suspension of MS₁₄

Present results in an experimental rat model confirm that the MS₁₄ possesses an anti-inflammatory activity that could inhibit the rat paw inflammation induced by formalin, especially in serial applications. The effectiveness of 150 mg/kg dose of the MS₁₄ was similar to or better than 10 mg/kg MPN and 300 mg/kg SS in these serial applications. MS₁₄ is a natural product that voluntarily is used by MS patients. Preliminary clinical studies indicated that MS₁₄ may have some benefits for improving quality of life

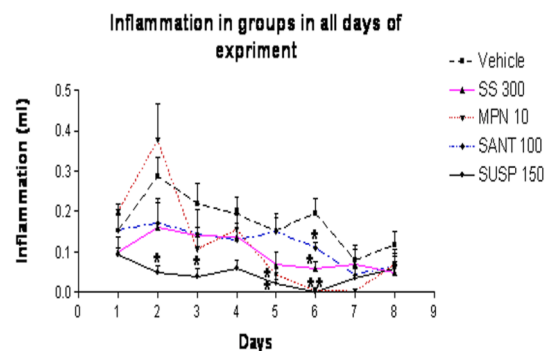


Fig 3. Effect of the MS₁₄ on paw edema induced by formalin in one week duration
Data represent mean $\pm$ S.E.M. for six rats.
In second and third day: * $P < 0.05$ compared with control group.
Fifth day: * $P < 0.05$, SUSP150 & MPN10 compared with control group.
Sixth day: * $P < 0.05$ in all administrations compared with control group.

and symptoms of the patients. MS₁₄ contains many organic salts, complexes and also trace elements such as Br, Sr, Va, Ti, Ni and Zn.²² Some studies have demonstrated the anti-inflammatory properties of some of these elements and MS₁₄ is an antioxidant agent with no observed adverse effects and is safe in chronic long term oral use.²¹ MS₁₄ contains *Apium graveolens* and *Hypericum perforatum*, two components that demonstrated anti-inflammatory activity in animal models and some of the effects of MS₁₄ in multiple sclerosis may have been due to the presence of these two plants.^{31,32}

In a previous study, it was found that oral treatment of the EAE mice with MS₁₄ not only halted the progression of the disease but also attenuated the inflammation in CNS, indicating that this herbal-marine compound has anti-inflammatory effects. Also this study showed anti-inflammatory properties for MS₁₄ in formalin test.²³

In conclusion, MS₁₄ possess anti-inflammatory effect in intra-peritoneal administration with dose dependent manner. This may suggest a possible mechanism for its action in MS.

To design more effective anti-inflammatory therapies for MS we need to understand in detail the different phases of the inflammatory process, in particular the timing and the kinetics of its detrimental and protective components. Therefore, further *in vitro* and *in vivo* experimental studies are needed to better understand mechanism of the active compounds of MS₁₄. Furthermore, their safety and efficacy for human diseases, including MS, need new randomized clinical trials.

List of acronyms

CNS – central nervous system
EAE - Experimental Allergic Encephalomyelitis
i.p. - intra-peritoneal
LSD - least significant difference
MPN - methyl prednisolon
MS - Multiple sclerosis
MS₁₄ - multiple sclerosis14
NSAIDs - nonsteroidal anti-inflammatory drugs
SANT - centrifuged
SUSP - suspension
SS - sodium salicylate

Contributions of Authors

MN, HE, MM, TT, ZB,NA,MA,ATMS,FE,PMM,AHN, and FG conception and design of the study, acquisition, analysis and interpretation of data, wrote the manuscript, performed literature review, article drafting and revision, reviewed and edited the manuscript critically, all authors approved the final version.

Acknowledgments

The authors would like to thank Farzan Institute for Research and Technology for technical assistance.

Funding

This research did not received any specific grant from funding agencies in the public, commercial, or not for profit sectors

Conflict of Interest

The authors declare no conflict of interests.

Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Corresponding Author

Farzaneh Ghaffari, School of Traditional Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Phone: +989123881874
ORCID iD: 0000-0002-5424-4348
E-mail: f_ghaffari@sbmu.ac.ir

E-mails and ORCID iD of co-authors

Mohsen Naseri: naseri@shahed.ac.ir
ORCID iD: . 0000-0001-9239-768X
Hossein Rezaeizadeh: hosseinrezaeizade@gmail.com
ORCID iD: 0000-0003-2703-9485
Mahdi Mirghazanfari: mmirghazanfari@gmail.com
ORCID iD: 0000-0001-9295-4482
Taiebeh Taheripana: panahtaheri@gmail.com
ORCID iD: 0000-0002-0651-1050
Zahra Bararpour: z.bararpour96@gmail.com
ORCID iD: 0000-0002-4466-9609
Neda Ashayeri: neda_ashayeri@yahoo.com
ORCID iD: 0000-0001-6619-511X
Majid Asghari: asghari.rall@gmail.com
ORCID iD: 0000-0003-1155-3172
Abbas Talebi Mazrae Shahi: a.talebi41@gmail.com
ORCID iD: 0000-0002-6021-2457
Fateme Emadi: fatemeh12emadi@yahoo.com
ORCID iD: 0000-0002-5053-4591
Parvaneh Mohseni Moghaddam:
p.mohseni@iautmu.ac.ir
ORCID iD: 0000-0002-0608-2305
Abbas Hashemi Nejad:
abbas.hasheminejad@shahed.ac.ir
ORCID iD: 0000-0001-9635-7669

References

1. Compston A, Coles A. Multiple sclerosis. Lancet. 2008 Oct 25;372(9648):1502-17. doi: 10.1016/S0140-6736(08)61620-7.
2. Lucchinetti CF, Popescu BF, Bunyan RF, Moll NM, Roemer SF, Lassmann H, Brück W, Parisi JE, Scheithauer BW, Giannini C, Weigand SD, Mandrekar J, Ransohoff RM. Inflammatory cortical demyelination in early multiple sclerosis. N Engl J Med. 2011 Dec 8;365(23):2188-97. doi: 10.1056/NEJMoal100648.
3. Matthews PM. Chronic inflammation in multiple sclerosis - seeing what was always there. Nat Rev

- Neurol. 2019 Oct;15(10):582-593. doi: 10.1038/s41582-019-0240-y.
4. Kidd PM. Multiple sclerosis, an autoimmune inflammatory disease: prospects for its integrative management. *Altern Med Rev*. 2001 Dec;6(6):540-66.
5. Zaffaroni M, Ghezzi A, Comi G. Intensive immunosuppression in multiple sclerosis. *Neurol Sci*. 2006 Mar;27 Suppl 1:S13-7. doi: 10.1007/s10072-006-0539-5.
6. Silbermann E, Senders A, Wooliscroft L, Rice J, Cameron M, Waslo C, Orban A, Chase E, Yadav V, Bourdette D, Spain RI. Cross-sectional survey of complementary and alternative medicine used in Oregon and Southwest Washington to treat multiple sclerosis: A 17-Year update. *Mult Scler Relat Disord*. 2020 Jun;41:102041. doi: 10.1016/j.msard.2020.102041. Epub 2020 Mar 7.
7. Crayton HJ, Rossman HS. Managing the symptoms of multiple sclerosis: a multimodal approach. *Clin Ther*. 2006 Apr;28(4):445-60. doi: 10.1016/j.clinthera.2006.04.005.
8. Moosavizadeh A, Ghaffari F, Mosavat SH, Zargaran A, Mokri A, Faghihzadeh S, Naseri M. The medieval Persian manuscript of Afyunieh: the first individual treatise on the opium and addiction in history. *J Integr Med*. 2018 Mar;16(2):77-83. doi: 10.1016/j.joim.2018.02.004. Epub 2018 Feb 5.
9. Ghaffari F, Naseri M, Jafari Hajati R, Zargaran A. Rhazes, a pioneer in contribution to trials in medical practice. *Acta Med Hist Adriat*. 2017 Dec;15(2):261-270. doi: 10.31952/amha.15.2.4.
10. Babaeian M, Naseri M, Kamalinejad M. The Efficacy of *Mentha longifolia* in the Treatment of Patients With Postprandial Distress Syndrome: A Double-Blind, Randomized Clinical Trial. *Iranian Red Crescent Med J* 2017;19(2); e34538.
11. Goshtasebi A, Mazari Z, Behboudi Gandevari S. Anti-hemorrhagic activity of *Punica granatum* L. flower (Persian Golnar) against heavy menstrual bleeding of endometrial origin: A double-blind, randomized controlled trial. *Med J Islamic Republic of Iran*. 2015; 29:199.
12. Ansari-pour M, Naseri M, Esfahani MM, Nabipour I, Rakhshani F, Zargaran A, Kelishadi R. Periconceptional care and offspring health at birth and long term, from the perspective of Avicenna. *J Integr Med*. 2019 Mar;17(2):80-86. doi: 10.1016/j.joim.2019.01.003. Epub 2019 Jan 11.
13. Nouri F, Zargaran A, Naseri M. Nutrition Therapy in Ancient and Medieval Persia. 2017;19(11): e13716.
14. Araj-Khodaei M, Noorbala AA, Yarani R, Emadi F, Emaratkar E, Faghihzadeh S, Parsian Z, Alijaniha F, Kamalinejad M, Naseri M. A double-blind, randomized pilot study for comparison of *Melissa officinalis* L. and *Lavandula angustifolia* Mill. with Fluoxetine for the treatment of depression. *BMC Complement Med Ther*. 2020 Jul 3;20(1):207. doi: 10.1186/s12906-020-03003-5.
15. Gorji A. Pharmacological treatment of headache using traditional Persian medicine. *Trends Pharmacol Sci*. 2003 Jul;24(7):331-4. doi: 10.1016/S0165-6147(03)00164-0.
16. Safari MB, Nozad A, Ghaffari F, Ghavamzadeh S, Alijaniha F, Naseri M. Efficacy of a Short-Term Low-Calorie Diet in Overweight and Obese Patients with Chronic Sciatica: A Randomized Controlled Trial. *J Altern Complement Med*. 2020 Jun;26(6):508-514. doi: 10.1089/acm.2019.0360. Epub 2020 May 20.
17. Ghaffari F, Naseri M, Movahhed M, Zargaran A. Spinal Traumas and their Treatments According to Avicenna's Canon of Medicine. *World Neurosurg*. 2015 Jul;84(1):173-7. doi: 10.1016/j.wneu.2015.03.011. Epub 2015 Mar 13.
18. Avicenna. The canon of medicine. Tehran: Soroush Press; 1997.
19. Hossaini-Tabib M. Tohfe of hakim Momen. Tehran and Qum: Mostafavi Press; 1959. (in Persian).
20. Aghili M. Makhzan-al-Advia. Tehran: Tehran University of Medical Sciences; 2009: 328.
21. Hajhashemi V, Ghafghazi T, Ahmadi A. Study the subacute toxicity of natural product MS14 in rat. *Daneshvar*, 2004; 50:11–14.
22. Naseri M, Ahmadi A, K Gharegozli K. A double blind, placebo-controlled, crossover study on the effect of MS14, an herbal-marine drug, on quality of life in patients with multiple sclerosis. *J Medicinal Plants Res*. 2009; 3(4):271-275.
23. Tafreshi AP, Ahmadi A, Ghaffarpur M, Mostafavi H, Rezaeizadeh H, Minaie B, Faghihzadeh S, Naseri M. An Iranian herbal-marine medicine, MS14, ameliorates experimental allergic encephalomyelitis. *Phytother Res*. 2008 Aug;22(8):1083-6. doi: 10.1002/ptr.2459.
24. Sadeghi AH, Ahmadi A. Cytotoxicity and antitumor properties of a marine compound, HESA-A, on cancer cells. *DARU J Pharmaceutical Sci*. 2003;11:82-87.
25. Ojewole JA. Antiinflammatory properties of *Hypoxis hemerocallidea* corm (African potato) extracts in rats. *Methods Find Exp Clin Pharmacol*. 2002 Dec;24(10):685-7. doi: 10.1358/mf.2002.24.10.80231
26. Agarwal RB, Rangari VD. Antiinflammatory and Antiarthritic activities of Lupeol and 19 a-H Lupeol isolated from *Strobilanthes callosus* and *Strobilanthes ixiocephala* roots. *Indian J Pharmacol*. 2003; 35: 384-387.
27. Dubuisson D, Dennis SG. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation

- in rats and cats. *Pain*. 1977 Dec;4(2):161-174. doi: 10.1016/0304-3959(77)90130-0.
28. Ahmadiani A, Javan M, Semnanian S, Barat E, Kamalinejad M. Anti-inflammatory and antipyretic effects of *Trigonella foenum-graecum* leaves extract in the rat. *J Ethnopharmacol*. 2001 May;75(2-3):283-6. doi: 10.1016/s0378-8741(01)00187-8.
 29. Sayyah M, Hadidi N, Kamalinejad M. Analgesic and anti-inflammatory activity of *Lactuca sativa* seed extract in rats. *J Ethnopharmacol*. 2004 Jun;92(2-3):325-9. doi: 10.1016/j.jep.2004.03.016.
 30. Lassmann H. Multiple sclerosis: is there neurodegeneration independent from inflammation? *J Neurol Sci*. 2007 Aug 15;259(1-2):3-6. doi: 10.1016/j.jns.2006.08.016. Epub 2007 Mar 23.
 31. Sosa S, Pace R, Bornancin A, Morazzoni P, Riva A, Tubaro A, Della Loggia R. Topical anti-inflammatory activity of extracts and compounds from *Hypericum perforatum* L. *J Pharm Pharmacol*. 2007 May;59(5):703-9. doi: 10.1211/jpp.59.5.0011.
 32. Emad AM, Ali SF, Abdel-Rahman EA, Meselhy MR, Farag MA, Ali SS, Abdel-Sattar EA. Anti-inflammatory and antioxidant effects of *Apium graveolens* L. extracts mitigate against fatal acetaminophen-induced acute liver toxicity. *J Food Biochem*. 2020 Jul 26:e13399. doi: 10.1111/jfbc.13399. Epub ahead of print.

Submission: October 5, 2021

Revision received: November 4, 2021

Accepted for publication: November 6, 2021