Anti-inflammatory activity of a natural herbal-marine drug (MS_{14} - SANT and SUSP) compared to sodium salicylate or methylprednisolone in a rat model for multiple sclerosis

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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 (MS_{14}). 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 MS_{14} (SANT 100 mg/kg) and suspension of MS_{14} (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 MS_{14} (100 mg/kg) was also significant only on the 6th day. SUSP MS_{14} (150 mg/kg) significantly reduced edema from second to 6th day. Intra-peritoneal injection of SUSP MS_{14} with 300 mg/kg was toxic, so excluded from the study. This research indicates that the MS_{14} possesses an anti-inflammatory effect after intra-peritoneal administration. Comparative anti-inflammatory effects of MS_{14} with Glucocorticoids in this study, may justify a possible mechanism for its action in multiple sclerosis, if further studies will provid strong statistically confirmatory effects in animals and safety human trials.

Key Words: Traditional Persian Medicine; *Penaeus latisculatus*; *Apium graveolens*; *Hypericum perforatum*; MS_{14}; anti-inflammatory action; *in vivo* animal experiments.

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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 none-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]

MS_{14} is an herbal-marine drug containing *Penaeus latisculatu*s (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 MS_{14} improve mobility and quality of life of patients.[22] Furthermore, in a rodent model study MS_{14} 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 MS_{14}, an experimental model in rats was designed to investigate the anti-inflammatory effects of MS_{14} in comparison to nonsteroidal anti-inflammatory drugs (NSAIDs) and Glucocorticoids.

Materials and Methods

Drugs

MS_{14} 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

MS_{14} contained 90% *Penaeus latisculatus* (King prawn), 5% *Apium graveolens L.* (Wild celery) and 5% *Hypericum perforatum L.* (St John's wort).

Preparation of MS_{14}

MS_{14} 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, intra-peritoneal (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 MS_{14} (SANT 100 mg/kg) and suspension of MS_{14} (SUSP 100, 150, 300 mg/kg) i.p., respectively.

Anti-inflammatorys 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 modificated 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 MS_{14}, of SS and of MPN are shown in Figure 1. The administration of MS_{14} with doses of 300 mg/kg, significantly inhibited the paw edema ($P < 0.05$ compared with control group).

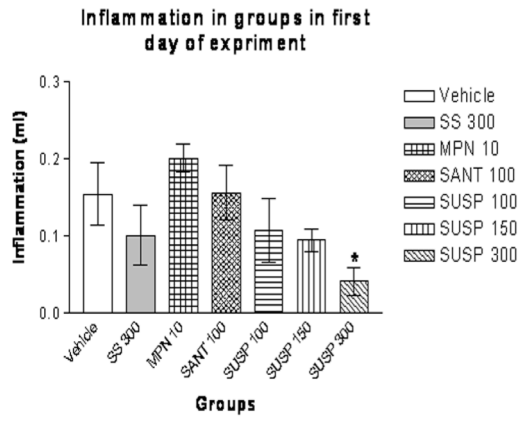

Fig 1. *Effect of the MS_{14} on paw edema induced by formalin*
Data represent mean ± S.E.M. for six rats.
∗P < 0.05 compared with control group.
SS: sodium salicylate; MPN: methyl prednisolon; SANT: centrifuged MS_{14}; SUSP: suspension of MS_{14}

Results of serial administration of different doses of MS_{14}, 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 MS_{14} (SANT 100 mg/kg) was also significant only in 6th day while MS_{14} (SUSP150 mg/kg) significantly reduced edema from second to 6th day.
Intra-peritoneal injection of MS_{14} 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 understoo, 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 MS_{14}. Thus, we evaluated the putative anti-inflammatory activities of the MS_{14} to clarify the mechanism of traditional belief in the relieving symptoms of multiple sclerosis by this compound.

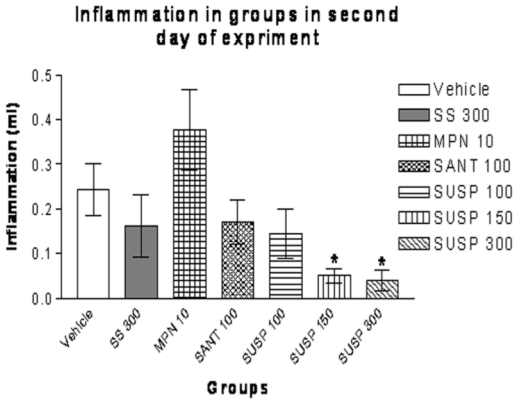

Fig 2. *Effect of the MS_{14} on paw edema induced by formalin*
Data represent mean ± S.E.M. for six rats.
∗P < 0.05 compared with control group.
SS: sodium salicylate; MPN: methyl prednisolon; SANT: centrifuged MS_{14}; SUSP: suspension of MS_{14}

Present results in an experimental rat model confirm that the MS_{14} 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 MS_{14} was similar to or better than 10 mg/kg MPN and 300 mg/kg SS in these serial applications.
MS_{14} is a natural product that voluntarily is used by MS patients. Preliminary clinical studies indicated that MS_{14} may have some benefits for improving quality of life

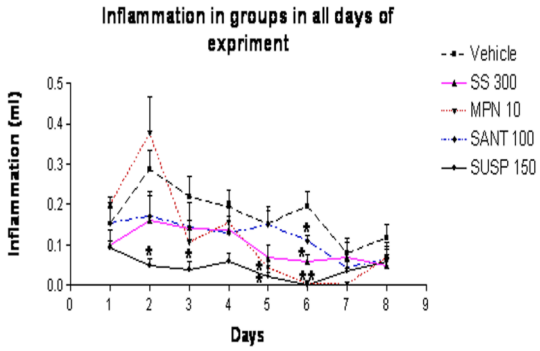

Fig 3. *Effect of the MS_{14} on paw edema induced by formalin in one week duration*
Data represent mean ± S.E.M. for six rats.
In second and third day: ∗P < 0.05 compared with control group.
Fifth day: ∗P < 0.05, SUSP150 & MPN10 compared with control group.
Sixth day: ∗P < 0.05 in all administrations

and symptoms of the patients. MS_{14} 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 MS_{14} is an antioxidant agent with no observed adverse effects and is safe in chronic long term oral use.[21] MS_{14} contains *Apium graveolens* and *Hypericum perforatum*, two components that demonstrated anti-inflammatory activity in animal models and some of the effects of MS_{14} 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 MS_{14} 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 MS_{14} in formalin test.[23]

In conclusion, MS_{14} 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 MS_{14}. 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
MS_{14} - 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.

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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.

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