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# Anti-inflammatory effect of the *Salvia sclarea* L. ethanolic extract on lipopolysaccharide-induced periodontitis in rats

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ABSTRACT

*Ethnopharmacological relevance:* *Salvia sclarea* L., clary, is an aromatic plant traditionally used in folk medicine for the treatment of various diseases and conditions. Although it has been primarily used as a stomachic, there are data on traditional use of *S. sclarea* as an agent against gingivitis, stomatitis and aphthae.
*Aim of the study:* The aim of the study was to examine the effect of the *S. sclarea* ethanolic extract on the lipopolysaccharide (LPS)-induced periodontitis in rats from the immunological and histopathological standpoint.
*Material and methods:* Periodontal inflammation in rats was induced by repeated injections of LPS from *Escherichia coli* into the interdental papilla between the first and second right maxillary molars. The extract was administered two times a day by oral gavage (200 mg/kg body weight). The inflammatory status was assessed by the measurements of proinflammatory cytokines interleukin-1$\beta$ (IL-1$\beta$), interleukin-6 (IL-6) and tumor necrosis factor-$\alpha$ (TNF-$\alpha$) of gingival tissues and descriptive analysis of histological sections of periodontium. Chemical characterization of the extract was determined using high performance liquid chromatography system (HPLC). Antioxidant activity of the extract was estimated with two *in vitro* complementary methods: 2,2-diphenyl-1-picrylhydrazyl and $\beta$-carotene/linoleic acid models.
*Results:* Treatment with *S. sclarea* extract, compared to the untreated group of the rats, significantly diminished the process of inflammation decreasing the levels of IL-1$\beta$, IL-6 and TNF-$\alpha$, reducing the gingival tissue lesions and preserving bone alveolar resorption. Considerably smaller number of inflammatory cells and larger number of fibroblasts was noticed. The administration of the extract three days earlier did not have significant preventive effects. Rosmarinic acid was the predominant compound in the extract. The extract showed strong antioxidant effects in both test systems.
*Conclusions:* *S. sclarea* extract manifested anti-inflammatory effect in LPS-induced periodontitis suggesting that it may have a role as a therapeutic agent in periodontal diseases. Having in mind that overproduction of reactive oxygen species is connected to periodontitis, the strong antioxidant capacity may be contributable to anti-inflammatory properties of the extract.

## 1. Introduction

Periodontal disease is a chronic infective disease of the periodontium caused by periodontopathic bacteria accumulated on the tooth surface, characterized by destruction of the tooth supporting tissues including alveolar bone resorption (Bentzen et al., 2005; Dumitrescu et al., 2004). Subgingival Gram-negative organisms containing lipopolysaccharides (LPS) cause inflammation of the periodontal tissues inducing a local response, which implies a polymorphonuclear leukocyte infiltration, a production of reactive oxygen species (ROS) and

*Abbreviations:* ANOVA, analysis of variance; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; DAD, diode array detector; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ELISA, enzyme-linked immunosorbent assay; GAE, gallic acid equivalents; HPLC, high performance liquid chromatography; HE, hematoxylin and eosin; $IC_{50}$, inhibitory concentration reducing 50% of radicals; IL-1$\beta$, interleukin-1$\beta$; IL-6, interleukin-6; LPS, lipopolysaccharide; PVPP, polyvinylpolypyrrolidone; ROS, reactive oxygen species; RANKL, receptor activator of nuclear factor kappa-B ligand; RA, rosmarinic acid; TNF-$\alpha$, tumor necrosis factor-$\alpha$; UV/VIS, ultraviolet–visible spectroscopy

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inflammatory mediators such as cytokines and prostaglandins, amplification of lytic enzymes and activation of osteoclasts, oedema and vascular dilatation (Page, 1991; Kjeldsen et al., 1993; Maruyama et al., 2011; Sculley and Langley-Evans, 2002). Recent investigations have shown that the gingival tissue from the inflamed area contains increased levels of some cytokines, primarily interleukin-1$\beta$ (IL-1$\beta$), interleukin-6 (IL-6), and tumor necrosis factor-$\alpha$ (TNF-$\alpha$) (Irwin and Myrillas, 1998; Stashenko et al., 1991; Vahabi et al., 2011), implying that these cytokines mainly participate in the pathogenesis of periodontitis (Agarwal et al., 1995). The culminating stadium of damaging effects of the produced cytokines and ROS is destruction of tooth-supported tissues including connective and mineralized tissue (Maruyama et al., 2011; Rogers et al., 2007).

A remarkable number of studies have demonstrated the use of plants to be a new trend in the prevention and treatment of periodontal diseases with less adverse effects on humans. Some drugs for oral inflammations treatment can change oral microbiota and cause teeth discoloration (Feres et al., 2015; Palombo, 2011; Sweetman, 2007). In addition, they can provoke gastrointestinal disturbances usually followed by diarrhea, nausea and vomiting, or rarely, cause phototoxicity and accumulation in bones and teeth (Sweetman, 2007). Therefore, the search for natural products or phytochemicals is growing increasingly with prospects to become the acceptable alternatives or complementary agents for oral inflammations (Palombo, 2011). Bioactive phytochemicals from herbs such as phenolic acids, flavonoids, tannins, terpenoids, alkaloids etc. have been found to be particularly helpful in periodontitis due to their potent antimicrobial and anti-inflammatory activities (Kumar et al., 2009). Plant species from the genus Salvia L., Lamiaceae family, especially Salvia officinalis L., are well-known effectual agents whose active components could reduce gingival inflammation, inhibit growth of plaques and have beneficial effects on the cavity prophylaxis (Ehrnhöfer-Ressler et al., 2013; Willershausen et al., 1991).

*Salvia sclarea* L. is a spicy, aromatic plant, known as anti-inflammatory, antimicrobial, antioxidant, antispasmodic, anticonvulsant, hypoglycemic and digestive agent (Moretti et al., 1997; Hammer et al., 1999; Miliauskas et al., 2004; Mantle et al., 2000; Leporatti et al., 1985). From herbal literatures, aqueous extracts of the plant have been used against various digestive disorders, as well as decoction and infusion for treating polyarthritis and acute rheumathism (Lawrence, 1994; Peana and Moretti, 2002; Rajagopal et al., 2013). Although there are data on traditional use of *S. sclarea* against gingivitis, stomatitis and aphthae (Leporatti et al., 1985), there have been no studies concerning its inhibiting impact on the development of periodontitis on an animal model; in addition this is the first experimental research focusing on anti-inflammatory properties of the *S. sclarea* extract. In this pilot study, we investigated the effectiveness of the extract of *S. sclarea* in suppressing the inflammation of the rats periodontium caused by LPS, by determining the levels of proinflammatory factors and histopathological analysis, as well as the chemical composition and antioxidant activity of the same extract.

## 2. Material and methods

### 2.1. Chemical reagents

All reagents and solvents in the investigations were of analytical or HPLC grade. Acetonitrile and methanol were purchased from J.T. Baker (Mallinckrodt Baker, Center Valley, PA) and chloroform from Carlo Erba Reagents S.A.S. (Val de Reuil, France). Ethanol was purchased from Zorka Pharma, Šabac, Serbia. Apigenin-7-*O*-glucoside, luteolin-7-*O*-glucoside and acacetin were obtained from Carl Roth (Karlsruhe, Germany) and butylated hydroxytoluene (BHT) from Supelco (Sigma Chemical Co., St Louis, MO, USA). Polyvinylpolypyrrolidone was obtained from Merck (Darmstadt, Germany). Caffeic acid and apigenin were purchased from Fluka (Sigma Chemical Co., St Louis, MO, USA). Lipopolysaccharide, Folin-Ciocalteu reagent, trifluoracetic acid, gallic acid, 2,2-diphenyl-1-picrylhydrazyl, rosmarinic acid, butylated hydroxyanisole (BHA), $\beta$-carotene, linoleic acid, luteolin and sodium carbonate were purchased from Sigma Aldrich (Sigma Chemical Co., St Louis, MO, USA).

### 2.2. Plant material

Aerial parts of *S. sclarea*, Lamiaceae family, in full flowering stage, were collected in the surrounding area of Nis, Serbia (GPS Coordinates: 43°33′05.27″ N, 22°03′06.26″ E; cca 334 m a.s.l.). Plant material was identified by Dr. Bojan Zlatkovic (Department of Biology and Ecology, Faculty of Science and Mathematics, University of Nis) and a voucher specimen has been deposited in the Herbarium of the Institute of Botany and Botanical Garden “Jevremovac”, University of Belgrade, Serbia (No. 17077).

### 2.3. Plant extraction

The plant material was primarily air-dried, protected from direct effect of sunlight and then milled to powder. The powdered material was extracted three times with 80% (v/v) ethanol (1:10) in an ultrasonic bath for 20 min. Final extract was obtained after filtration through Watman paper and total removal of the solvent in a rotary vacuum evaporator at 40 °C. The average yield of extraction was 15.31 ± 1.73%. Prior to the experiment, the solid extract was kept in a sealed bottle in a dark place at 4 °C.

### 2.4. Experimental design with animals

All experimental procedures were approved by the Ethical committee of Medical faculty, University of Nis (No 01-9002-03) and were performed in accordance with Good Scientific Practice. Thirty male Wistar rats, obtained from vivarium of Medical faculty in Nis, ten weeks old (247.5 ± 54.16 g), were kept in wire cages, in a room with proper, controlled conditions of temperature (21–23 °C) and humidity (65–70%) with 12 h light/dark cycle. The animals were fed with milled pellets, and had free access to water during the experiment. Rats were anaesthetized with a 10% ketamin-hydrochloride solution (2 mL/kg) administered intraperitoneally. Lipopolyssacharide, derived from *Escherichia coli* (055:B5) and diluted in a sterile saline solution, was used for induction of periodontitis. One microliter of LPS solution (10 μg/μL) was slowly injected into interdental papilla between the first and second right maxillary molar (Dumitrescu et al., 2004), two times for ten days, using a Hamilton microsyringe.

Rats were randomly divided into five experimental groups with six animals in each: (I) injected with saline, treated with vehicle (distilled water); (II) injected with saline, treated with *S. sclarea* extract; (III) injected with LPS, treated with vehicle; (IV) injected with LPS, treated with *S. sclarea* extract; (V) injected with LPS, treated with *S. sclarea* extract three days before LPS injection and during the period of examination. Groups I and II received the same volume (1 μL) of sterile saline injection in the same location of rat maxilla. Groups II, III, IV and V received *S. sclarea* extract dissolved in water which was administered two times daily by oral gavage (200 mg/kg body weight). Administration of dissolved extracts or vehicle started the same day when injections were given except V group which was treated with the extract preventively, three days before LPS injections.

After ten days, the rats were sacrificed by an overdose of anesthetic ketamine, then the maxillae were isolated and hemisected. The soft tissues around the molars of rats were carefully anatomized and prepared for biochemical analysis. Some of the sections from each group including molars with their encircling tissues were subjected to a standard histological procedure.