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The effect of *Nigella sativa* oil on prevention of myringosclerosis in a Guinea pig model

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ABSTRACT

Objectives: In this study, our aim was to identify the possible effects of *Nigella sativa* L. (NS) [blackcumin] seed oil on the prevention of experimentally induced myringosclerosis (MS).
Materials and methods: Fourteen Guinea pigs were used and they were divided into three groups. Tympanic membranes (TM) of all animals were perforated and then group I was treated with saline soaked gel foams as a control group, group II was treated with 0.5 ml NS oil soaked gel foams at 0, 24 and 48 h and group III was treated with 5 ml NS oil orally at 0, 24, 48, 72 and 120 h. After 15 days, all animals were euthanized. Tympanic membranes were evaluated macroscopically and histopathologically.
Results: Groups I showed extensive myringosclerosis in contrast to those of Groups II and III which had significantly less changes ($p < 0.05$). The fibrosis and inflammation in the lamina propria of the tympanic membranes of Groups I was found to be significantly more pronounced ($p < 0.05$). The tympanic membranes were found to be significantly thinner in Groups II and III when compared with Groups I ($p < 0.05$).
Conclusions: The results of this study suggested that topical or oral administration of NS oil supressed the inflammation and fibroblastic activity in the lamina propria of the myringotomized TMs of the Guinea pigs. For providing further evidence to use plant extracts as antioxidant and antiinflammatory therapy after myringotomy or ventilation tube insertion, further clinical studies with larger population will be essential.

1. Introduction

Myringosclerosis (MS) is an irreversible degenerative pathology affecting tympanic membrane (TM) and synchronous dystrophic calcification occurs in the fibrous layer [1]. Generally, MS is caused by myringotomy, ventilation tube insertion, trauma, chemical agents, chronic middle ear effusion and infection, and autoimmunity [2]. It is histopathologically characterized by hyaline degeneration, calcification, and rise in collagen fiber in the lamina propria (LP) of the TM [3]. In addition, as calcium and phosphorus are accumulated on this structure, crystallization and sclerosis will occur [2].

In paediatric population, otitis media with effusion is a common disease and often necessitates a surgical intervention like myringotomy or ventilation tube insertion which leads to a common sequel, myringosclerosis (MS). The exact pathogenesis of MS is not known very well. In previous studies, the course of MS has been investigated by making a TM injury with trauma or paracentesis [4]. These studies have shown that traumatic perforations or formation of oxygen derived free radicals because of hyperoxidative conditions may be the primary reason in the formation of MS [4,5]. Therefore, MS may be reduced or prevented by inhibiting the harmful effects of oxygen derived free radicals and administration

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of anti-inflammatory or antioxidant agents. Starting from this point of view, for minimizing the formation of MS after experimental paracentesis, several forms of free radical scavengers have been used in previous reports in the literature [3,4,6–10].

Nigella sativa L. (NS) is a plant from the family Ranunculaceae [11]. It is also known as black seed or black cumin in English [12]. The seeds using for medicinal purpose are the most important parts of this plant. They contain fixed oil, volatile oil, alkaloids, proteins, and saponins [11]. Primarily Thymoquinone (TQ) and other active constituents of NS have recently been reported to possess several potential therapeutic properties; antimicrobial, analgesic, antidiabetic, anticancer, anti-inflammatory, antioxidant and immunomodulator [11]. And also it has been evaluated in clinical trials with various health conditions such as, hyperlipidemia, diabetes mellitus, hypertension, asthma, allergy, cough, bronchitis, fever, headache, infertility, rheumatoid arthritis and gastrointestinal diseases [13].

In this study, the purpose was to investigate the possible preventive effect of oral and topical forms of NS oil on the development of MS in the myringotomized TMs of the Guinea pigs using histopathology and otomiscroscopy techniques.

2. Materials and methods

2.1. Animal maintenance and experimental material

This study was approved by the Animal Experiments Ethic Committee of Yeditepe University (YUDHEK) (prot.no:01.11.2016–505). Fourteen Guinea pigs, male, weighing 400–600 g, at age 16–18 weeks, bred in Yeditepe University Animal Experiments Laboratories were used in this study. The animals were kept in ordinary cages and can access freely to food and water with artificial lighting from 8:00 a.m. to 8:00 p.m. at a relatively humidity of 50 ± 10% and a temperature of 23 ± 3 °C. Before and after the surgery, if any signs of external and middle ear infection in the animals was detected, those were excluded from the study.

NS oil was administered topically and orally in two groups of animals. Cold pressed NS oil [Black Cumin Seed Oil- *Nigella sativa* Oleum preparation] was provided by Zade Vital Natural Supplements, Konya, Turkey. The composition of the Nigella sativa seed oil, which was used in the present study was determined by GC-MS technique and found to be as follows: Linoleic acid (57.537%), oleic acid (23.851%), palmitic acid (11.75%), stearic acid (3.086%), cis-11,14-eicosapentaenoic acid (2.413%), gondoic acid (0.33%), linolenic acid (0.247%), arachidic acid (0.194%), palmitoleic acid (0.171%), myristic acid (0.139%), erusic acid (0.085%), heptadecanoic acid (0.064%), cis-10-heptadecanoic acid (0.044%), behenic acid (0.030%), cis-13,16-docosahexaenoic acid (0.037%), lignoceric acid (0.023%).

2.2. Experiment designing and surgical procedure

All animals were randomly divided into three groups and two groups include five and one group includes four male Guinea pigs. Animals were anesthetized with intramuscular injection of xylazine hydrochloride (5 mg/kg) and ketamine hydrochloride (50 mg/kg). A sterile perforation with a 0.5 mm pick through an ear speculum was performed in the superior posterior quadrant of the TMs in bilateral ears. In group I, a gelfoam particle soaked with saline solution was applied on the perforation of both TMs of animals as a control group. Animals in group II had treated topically with a gelfoam particle soaked with NS oil immediately after myringotomy, and 0.5 ml NS oil was dropped into the ear in the 24th and 48th hours [3,4,6]. In group III, 5 ml NS oil solution were given orally five times; immediately after myringotomy, in the 24th h (day-1), 48th h (day-2), 72nd h (day-3), and 120th h (day-5). We administered high doses compared to previous human studies because minimal toxic effect of NS oil have been reported previously [11]. We researched the preventive effect of NS oil via using consecutive high doses to obtain optimum systemic effect in a short time. Dose ranging studies were not performed because this was a preliminary study to show the preventive effect of NS oil. The dose ranging study is also needed as a secondary issue.

2.3. Otomicroscopic examination

Otomicroscopic examination was performed on the 15th day of the experiment after all animals were euthanized with carbon dioxide. The author who scored the degree of tympanic membrane inflammation was blinded to the treatment group of the animals being examined. The extent of MS in the pars tensa of the TMs was evaluated semiquantitatively as follows: (0) if no visible MS; (+) if there was white halo around umbo; (++) if there was white halo around umbo and white line beside the handle of the malleus and along the annulus; and (+++) if there was a confluent whitish deposit forming a horseshoe pattern [14].

2.4. Histopathological evaluation

After the otomicroscopic evaluations were completed, tympanic bulla was opened by microdissection and TM, its surrounding bony annulus, and cochlea were removed together. The specimens were fixed in 10% buffered formaldehyde solution overnight and then decalcified with ethylenediamine tetra-acetic acid for 10 days. All specimens were cut into two pieces from the same region and at the same angle through a line bisecting the perforation. Both pieces were embedded in parafine and sectioned in 5 μm thickness. Four serial sections were taken from each parafine block (two were stained with Masson's Trichrome (Bio-optica, Milan, Italy) and two were stained with Hematoxylin & Eosin staining.) to evaluate the inflammation, fibrosis, and thickness of the TMs in each animal. For examining the collagen fibers and sclerotic changes in the LP of TMs, Masson's Trichrome staining was used. Sclerotic areas were stained with Masson's Trichrome staining and appeared as blue areas rather than white. Also, calcification was irregular bordered areas that appears as dark purplish pink color in H&E staining. For measuring the thickness of the TM, the healed perforation area of the TM (i.e. the area where inflammation occurs) observed in the sections was evaluated.

Evaluation of all specimens was done by a pathologist blinded to treatment types given to each group. Histopathologically, all the micro measurements were done quantitatively on the digital image output that was taken by the Olympus DP73 camera by using the software program of the same digital microscope and the intensity of the fibrosis degree, inflammation, and the thickness of the TMs were evaluated using a digital light microscope Olympus BX53. Then the fibrosis degree and the sclerotic changes in the LP were scored as: (0) for no fibrosis; (1) for mild fibrosis; (2) for moderate fibrosis and (3) for marked fibrosis and sclerosis and the intensity of the inflammation was scored as: (0) for no inflammation; (1) for mild inflammation; (2) for moderate inflammation; and (3) for marked inflammation, intense exudation and granulation [4].

2.5. Statistical analysis

Statistical analyses were performed using SPSS v.21 (SPSS, Inc. Chicago, IL, USA) and a value of $p < 0,05$ was accepted as significant. Mean thickness of the TMs were compared with Mann Whitney *U* test and otomicroscopic MS scores and inflammation in the LP in each group were compared with Pearson Chi-Square test.