Comparing the Effect of Myristica fragrans and Flunixin on Adjuvant-Induced Arthritis in Rat

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Abstract

Background: Nutmeg, Myristica fragrans Houtt, has shown anti-inflammatory properties in some studies. At present experimental study, we evaluated the effect of seed extract of nutmeg on adjuvant-induced arthritis in rats in comparison with flunixin meglumine.

Materials and Methods: Experimental study was done in six groups of Wistar rats (each group 8 rats) as following: Group 1 was kept as control under similar conditions to other groups. All other rats received complete Freund's adjuvant at dose 0.1 ml which injected under skin of foot. Group 2 was received vehicle (normal saline). Group 3 received flunixin intraperitonealy at dose of 2 mg/kg body weight of rats daily for 12 days. Group 4 to 6 received extract of nutmeg at dose 100, 200 and 300 mg/kg intraperitonealy and daily for 12 days. Four rats in each group were anesthetized and blood collected for serum analysis on 12th day. The ankle joint prepared for histopathological examination. The remained rats were kept until 21th day. Levels of the cytokine TNF-α in serum was measured using ELISA kit.

Results: The serum levels of TNF-α in the group 2 were significantly increased; while nutmeg decreased the elevated TNF-α level in a dose-dependent manner but significantly with 300 mg/kg. The flunixin did not significantly decrease the levels of TNF-α. Nutmeg treated rats manifested pathological events in the ankle joints to a markedly lesser degree. Flunixin prevented pannus formation but it was ineffective in other lesions.

Conclusion: Thus, nutmeg protected the joints against cartilage destruction and bone erosion in a dose-dependent manner.

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory and destructive joint disease that affect on life quality [1, 2]. It is targets the synovial membrane of joints but that can also have systemic signs [3]. Although the causes of RA remain unknown, some studies have shown the key roles of cytokines, such as tumor necrosis factor- α (TNF- α), interleukin-1β (IL-1 β), and IL-6 in the pathogenesis of RA [4, 5]. These cytokines are present in synovial fluid and joint [4]. Also these cytokines are produced through activation of T cells in RA [6, 7].

Adjuvant arthritis is an experimental disorder and occurs 10-14 days after inoculation of the animal with a suspension of adjuvant. Mycobacterial cell wall material (i.e., complete Freund's adjuvant) has been used as the adjuvant in most studies. It can be suppressed by nonsteroidal, antiinflammatory drugs, corticosteroids, or immunosuppressive agents [1-5, 8, 9].

Mace is the fleshy orange–red aril around the seed of nutmeg, Myristica fragrans Houtt, which belongs to the family myristicaceae [10]. The potential anti-cancer properties of mace including trans mammary modulation of xenobiotic metabolizing enzymes in the liver of mouse pups [11], prevention of DMBA (7, 12 dimethyl benzanthracene)-induced papillomagenesis in the skin of mice [12] and its modulatory effects on hepatic detoxification have been reported [13]. A number of pharmacological studies show properties of mace such as anti-inflammatory properties of myristicin from mace, antifungal, antibacterial, larvicidal and antioxidant potential [9, 14-17].

Seeds of Myristica fragrans Houtt are psychotomimetic and possess both stimulatory and depressant activities. Nutmeg, also possesses carminative, astringent, hypolipidaemic, antithrombotic, antiplatelet aggregation, antifungal, aphrodisiac, and anti-inflammatory activities. Fixed oil contains myristicin, myristic acid while volatile oil contains pinene, sabine, camphene, elemicin, isoelemicin, eugenol, methoxyeugenol, isoeugenol, safrole, etc. Eugenol, the major constituent of volatile oil inhibits lipid peroxidation and maintains activities of superoxide dismutase, catalase, glutathione peroxidase, glutamine transferase, and glucose 6-phosphate dehydrogenase. Chloroform extract of nutmeg showed analgesic and anti-inflammatory activity in rodents. Although the anxiogenic principle may not find any use in
therapeutics, it can be a useful research tool in experimental pharmacology [18]. Flunixin meglumine is a non-steroidal anti-inflammatory agent. It is used for treatment of inflammatory conditions especially visceral inflammations and pain. Flunixin meglumine provided analgesia in amphotericin B-induced synovitis-arthritis in animal model [19]. This important property (visceral anti-inflammatory) of flunixin as a nonsteroidal anti-inflammatory agent conducted us to evaluation its effect on RA. At present experimental study, we evaluated seed extract of nutmeg effect on adjuvant-induced arthritis in rats in comparison with flunixin meglumine.

Materials and Methods

In this experimental study the seed of nutmeg was purchased from Ahvaz herbal shop and authentication of the seeds was done in agriculture department of Shahid Chamran University of Ahvaz. The hydro-ethanolic extract was prepared by maceration method and filtrated. The 48 male Wistar rats were purchased from laboratory animal center of Jundishapour university. The age of rats was 7 weeks. The animals access to food and water ad libitum under 12 h light and 12 h dark conditions. The animal care was provided under the supervision of a qualified veterinarian.

This experimental animal model study was done in six groups of rats (each group 8 rats) as following: Group 1 was kept as control under similar conditions to other groups. All other rats received complete Freund's adjuvant (Difco, Germany) at dose 0.1 ml which injected under skin of foot. Group 1 intraperitoneally received normal saline (as vehicle) daily for 12 h days. Group 2 was kept without drug. Group 3 intraperitoneally received flunixin meglumine (Razak Co., Iran) at dose 2 mg/kg body weight of rats daily for 12 days. Group 4 to 6 received extract of nutmeg at dose 100, 200 and 300 mg/kg intraperitoneally and daily for 12 days. Four rats in each group were anesthetized by ketamine hydrochloride and blood collected for serum analysis on 12th day. The ankle joint of these rats prepared for histopathological examination. Other rats were kept until 21th day. Levels of the cytokines TNF-α in blood serum was measured using commercially available ELISA kit for TNF-α (Ebioscience) according to the manufacturer's recommendations.

The rats were sacrificed by ketamine hydrochloride and the hind paws were excised and placed in 10% PBS-buffered formalin. The fixed tissues of the ankle joints were then decalcified in formic acid, longitudinally cut into 5 micron sections, and stained with hematoxilin and eosin (H&E). Grading of cellular infiltration (polymorphonuclear cells, macrophages or lymphocytes), joint space narrowing, synovial hyperplasia, pannus formation, and bone and cartilage erosion of the ankle joints were examined by light microscope.

This grading was done by using a semiquantitative scale from 0 (normal), 1 (mild changes), 2 (moderate changes), and 3 (severe changes) based on pathological examination was done by Cai et al.

Data of serum TNF-α level were expressed as means±SEM. One-way analysis of variance with post hoc LSD test (SPSS-16, SPSS Inc., Chicago, IL, USA) was used to analyze differences between different groups. Statistical significance was accepted for p<0.05.

Results

Levels of cytokine TNF-α in the blood serum were determined on days 12 and 21 after arthritis induction. Consistent with the joint swelling, the mean of serum TNF-α was significantly increased in the vehicle-treated rats and reached to 141 pg/ml. However, this mean was 18.5 pg/ml in normal rats (without arthritis). Treatment with nutmeg decreased the elevated TNF-α by dose-dependent effect (Fig. 1). Nutmeg at dose of 300 mg/kg administered from the day of arthritis induction (day 0) significantly (p<0.05) reduced serum TNF-α levels. The flunixin did not significantly decrease the elevated serum TNF-α concentration (Fig. 1).

The histological sections of the ankle joints from normal, vehicle-treated, and nutmeg-treated rats are shown in figures 2-4. Histopathological examination of the synovial joints of the vehicle-treated rats revealed marked cellular infiltration, synovial hyperplasia, and joint space narrowing. Severe pannus formation, bone, and cartilage destruction were also observed in the synovial joints of vehicle-treated rats as compared to the synovial joints of normal rats. Nutmeg treated rats manifested these pathological events in the ankle joints to a markedly lesser degree. Further histopathological scoring demonstrated that treatments with nutmeg, on both days 12 and 21 after arthritis induction, protected the joints against cartilage destruction and bone erosion and other histopathological changes in a dose-dependent manner, but better with 300 mg/kg. Flunixin also decreased histopathological changes especially prevented pannus formation, but its effect lesser than nutmeg at dose 300 mg/kg.

![Figure 1. Means±SE of serum TNF-α levels of rats; 1-5: groups of rats received adjuvant, 2: group received flunixin, 3-5: groups received nutmeg at dose 100, 200 and 300mg/kg respectively. *represent significant difference at p<0.05](image-url)
Figure 2. Illustration histopathological sections of the ankle joints of rats in control group. Flashes show cubical cells, stares show fibroblasts and triangles show fatty cells (H&E×40).

Figure 3. A. Illustration histopathological sections of the ankle joints of rats in group 2 received Freund’s adjuvant and vehicle after 12 (up) and 21 (down) days. Stare shows polymorphonuclear cells and acute inflammation in left figure (H&E×40) and B. fibroblasts. Triangle shows giant cells in right figure (H&E×20).

Figure 4. Illustration histopathological sections of the ankle joints of rats in group 2 received Freund’s adjuvant and nutmeg (200 mg/kg) after 21 days. Stare shows normal surface of joint and triangle shows decreasing.

Discussion

The results show injection of Freund’s adjuvant produces experimental arthritis and elevates serum TNF-α as well as induces significant histopathological changes in ankle joints. The hydro-ethanolic extract of nutmeg has preventive and protective effect on changes of serum TNF-α and joint tissue. This extract decreased signs and inflammation in ankle joints.

RA is a chronic inflammatory and systemic autoimmune disease characterized by a number of inflammatory and destructive events such as joint pain and swelling, synovial hyperplasia, pannus formation, cartilage and bone destruction, and joint malformation [20]. Many cell populations, various cytokines, and different inflammatory mediators are involved in the generation of the pathological events characteristic of RA [21]. At present animal model study, we induced RA and demonstrated pathological changes and the level of TNF-α was elevated in vehicle-treated rats. These finding are similar to other related studies [4, 9, 22]. The inflammatory reactions especially immunological mediators such as cytokines seem to have key role at this process [5, 7]. Flunixin is anti-inflammatory agent without immunomodulatory effect, thus it could not prevent elevation of serum TNF-α levels. Therefore it cannot effective rheumatoid conditions. The effect of flunixin on RA was not evaluated by other researchers. However, indomethacin as a nonsteroidal anti-inflammatory agent had significant antirheumatoid effect in some studies including study was done by Cai et al. [9].

The extract of nutmeg had dose-dependent antirheumatoid effect. This efficacy may be related its immunomodulatory effect. This extract prevents both pathological changes and TNF-α elevation. This means this agent can penetrate in joint. Nutmeg shows antioxidative effect [17], the oxidative stress mediates rheumatoid pathogenesis [20], and thus it seems the effectiveness of nutmeg at present study may also relate to its antioxidative effect. Hocking et al. demonstrated...
flunixin is useful in treatment of inflammatory pain in domestic fowl using the microcrystalline sodium urate model of articular pain [23]. In conclusion, the present study indicates that nutmeg not only controls arthritis and the inflammation associated with joint synovitis, but also prevents cartilage and bone destruction of the arthritic joints of rats. Thus, Nutmeg may have potential for the further investigation in other animal models and clinical trials.

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**References**

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The authors declare no conflict of interest.

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