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# Resveratrol, a natural antioxidant, protects monosodium iodoacetate-induced osteoarthritic pain in rats

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ABSTRACT

*Background:* Osteoarthritis (OA) is a chronic progressive joint disease characterized by advanced joint pain, subchondral bone sclerosis and articular cartilage degeneration. Resveratrol has been shown to have anti-inflammatory, cardioprotective and antioxidant properties and to inhibit platelet aggregation and coagulation. However, the effects of resveratrol on OA have not been examined. In this study, we investigate the protective effects of resveratrol on monosodium iodoacetate (MIA)-induced OA through inhibition of cyclooxygenase (COX-2) and inducible nitric oxide synthase (iNOS) signaling pathway in a rat model.

*Methods:* A single intra-articular injection of MIA was injected into rats for the induction of OA. The mechanical, heat and cold hyperalgesia were measured at days 0, 7 and 14. The serum and synovial fluid levels of IL-1β, IL-10 and TNF-α and osteocalcin were measured by enzyme-linked immunosorbent assay. The mRNA and protein expressions of IL-1β, IL-10, TNF-α, Il-6, MMP-13 and COX-2 and iNOS were determined by RT-PCR and western blot, respectively. Osteoarthritic lesion in the knee joint was evaluated by histological analysis.

*Results:* MIA-injected rats treated with resveratrol at a dose of either 5 or 10 mg/kg body weight were significantly reduced hyperalgesia of mechanical, heat and cold and increased the vertical and horizontal movements. Subsequently, MIA-injected rats increased serum and synovial fluid levels of IL-1β, IL-10, IL-6, TNF-α, MMP-13 and osteoclastic activity marker, osteocalcin and its articular cartilage mRNA and protein expressions. Further, MIA-injected rats increased COX-2 and iNOS mRNA and protein expressions were decreased by resveratrol. The protective effect of resveratrol was comparable to a reference drug, etoricoxib. The cartilage damage induced by MIA were attenuated by resveratrol.

*Conclusions:* Taken together, resveratrol has the potential to improve MIA-induced cartilage damage by inhibiting the levels and expressions of inflammatory mediators suggesting that resveratrol may be a potential therapeutic agent for OA.



## 1. Introduction

Osteoarthritis (OA) is a chronic degenerative joint disease and the major common type of arthritis characterized by advanced joint pain, articular cartilage degeneration and subchondral bone sclerosis [1,2]. The extracellular matrix proteins including collagen, proteoglycans and hyaluronic acid degraded by articular inflammation are associated with progression of OA [3]. Among cytokines that are involved in the advancement of OA, tumor necrosis factor (TNF)-α and interleukin (IL) are the predominant cytokines contributed in the physiopathology of OA [4–7]. Furthermore, articular inflammation is directly associated with cartilage degradation that increases mediators and molecules including inducible nitric oxide synthase (iNOS), IL-8 and IL-6 [8]. Therefore, identifying drugs that inhibit proinflammatory cytokines, leads to suppression of inflammation, which may potentially be useful for OA therapeutic strategies. Despite the anti-inflammatory drugs (NSAIDs) are available for the treatment of OA, these drug efficacy is relatively limited and it has severe adverse effects. Recently, research has been focused on natural products from plants that have multifunctional anti-inflammatory effects that may decrease the threat of toxicity and opposing effects of the drug the treatment of OA.

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**Table 1**
Experimental design.

| Group | Treatment & Schedule |
|---|---|
| Control | Rats were injected with 50 μl saline on day 0 and oral administration of vehicle (DMSO) from day 0 to 14 daily. |
| MIA + Vehicle | Single dose of 3 mg of monosodium iodoacetate (MIA) were injected into right knee joint cavity and followed by oral administration of vehicle (DMSO) from day 0 to 14 daily. |
| MIA + RESV (5) | Single dose of 3 mg of monosodium iodoacetate (MIA) were injected into right knee joint cavity and followed by oral administration of resveratrol at 5 mg/kg b.w from day 0 to 14 daily. |
| MIA + RESV (10) | Single dose of 3 mg of monosodium iodoacetate (MIA) were injected into right knee joint cavity and followed by oral administration of resveratrol at 10 mg/kg b.w from day 0 to 14 daily. |
| MIA + ETOR | Single dose of 3 mg of monosodium iodoacetate (MIA) were injected into right knee joint cavity and followed by oral administration of etoricoxib at 10 mg/kg b.w from day 0 to 14 daily. |

Resveratrol (trans-3,5,4′-trihydroxystilbene), a stilbene found in plants and fruits such as grapes, berries and peanuts [9]. Resveratrol has been shown to have anti-inflammatory, cardio-protective and antioxidant properties and to inhibit platelet aggregation and coagulation [10–12]. In addition, resveratrol has significant chemopreventive effect in several cancer models [11,13,14]. In vivo animal studies suggest that resveratrol protects high calorie diet-induced diseases and improves mitochondrial function [15,16]. Further, studies has been demonstrated that resveratrol have significant beneficial effect on atherosclerosis [17] and alzheimer's disease [18]. Several studies suggest that resveratrol exerts anti-osteoarthritis effects through inhibitions of apoptosis, inflammation and oxidative functions *in vitro* [19] and *in vivo* for OA including rabbit [20–24]. However, it is not clear whether resveratrol can inhibits COX-2 and iNOS expression through suppression of tumor necrosis factor (TNF)-α and interleukin and in a rat model. Therefore, the purpose of this study was to examine the defensive effects of resveratrol on monosodium iodoacetate (MIA)-induced OA through inhibition of COX-2 and iNOS signaling pathway in a rat model. The efficacy was compared with anti-inflammatory drug, etoricoxib.

## 2. Materials and methods

### 2.1. Chemicals

Monosodium iodoacetate (MIA) and Resveratrol (3,5,4′-trihydroxy-tran-stilbene) were procured from Sigma Chemical (Sigma-Aldrich, St. Louis, MO, USA). All the other chemicals and reagents were procured from local companies.

### 2.2. Animals

Male Sprague-Dawley rats of 2 months old age (200–230 g) were obtained from the Shanghai SLAC Laboratory Animal Co. Ltd. The rats were maintained in solid bottom cages with standard chow with free access of water at constant room temperature (22 °C) and relative humidity of 40 – 60% and a 12 h light/dark cycle. All animal procedures were carried out accordance with Institutional Animal care and use Committee guidelines. The ethical approval for the experiment was obtained from the Research and Ethics Board, Zhengzhou Orthopedics Hospital affiliated to Henan University, Zhengzhou, China (Ref no. 2320339). We have followed the ARRIVE guidelines for reporting animal research [25]. A completed ARRIVE guidelines checklist is included.

### 2.3. Induction of OA

The OA induction protocol was followed as described previously [26]. The rats were divided into five groups and each group comprising of 5 rats as summarized in Table 1. The right knee of the rats were shaved and disinfected with 70% alcohol after anaesthetized with xylazine (10 mg/kg) and ketamine hydrochloride (50 mg/kg). On day 0, all groups of rats except control group, a single intra-articular injection of 50 μl of MIA (3 mg, dissolved in saline) were administered into right knee joint by infrapatellar ligament using a 300 μl syringe fitted with a 29 G needle. From day 0–14, rats were orally administered daily with vehicle (DMSO) or 5 or 10 mg/kg of resveratrol or 10 mg/kg of etoricoxib. All rats were weighed and observed every other alternate days to assess knee

**Table 2**
RT-PCR primers.

| Gene Name | Primer Sequences (5′-3′) | Product length (bp) | Annealing Temperature (sec) |
|---|---|---|---|
| IL-1B | Forward-TGA TGT TCC CAT TAG ACA GC<br>Reverse- GAG GTG CTG ATG TAC CAG TT | 378 | 60 °C (30 s) |
| IL-10 | Forward-CAG TCA GCC AGA CCC ACA T<br>Reverse- GCT CCA CTG CCT TGC TTT | 322 | 66 °C (45 s) |
| TNF-a | GTA GCC CAC GTC GTA GCA AA<br>Reverse- CCC TTC TCC AGC TGG AAG AC | 346 | 58 °C (30 s) |
| iNOS | Forward TTC TTT GCT TCT GTG CTT AAT GCG<br>Reverse- GTT GTT GCT GAA CTT CCA ATC GT | 1061 | 65 °C (45 s) |
| COX-2 | Forward CTG CAT GTG GCT GAT GTC ATC<br>Reverse- AGG ACC CGT CAT CTC CAG GGT AAT C | 1061 | 65 °C (45 s) |
| GAPDH | Forward TGGTGAAGGTCGGTGTGAAC<br>Reverse- TTCCCATTCTCAGCCTTGAC | 258 | 60 °C (30 s) |